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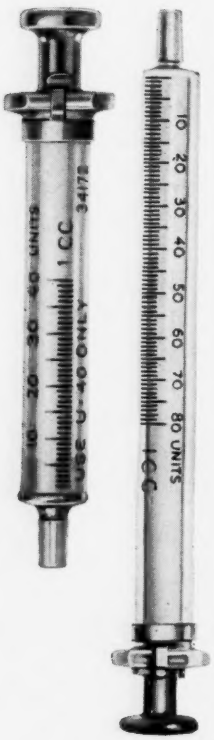

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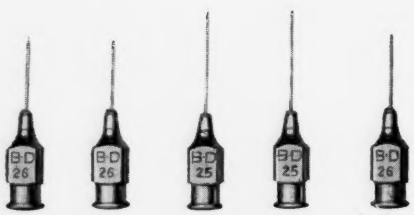

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The Clinical Society of the New York Diabetes Association, an Affiliate of the American Diabetes Association, Fourth Symposium Day on Diabetes Mellitus

Insulin, Glucagon and the Oral Hypoglycemic Sulfonylureas

HAROLD BRANDALEONE, M.D., (*Chairman, Clinical Society, New York Diabetes Association*): On behalf of the Clinical Society of the New York Diabetes Association, I wish to welcome you to this Fourth Symposium day. As you know, our Society has assumed the responsibility of arranging these Symposia as part of our professional education program. This year, the oral sulfonylureas have stimulated the imagination of those interested in diabetes. Irrespective of the final value of the sulfonylureas as therapeutic agents, the studies of these preparations have opened a new avenue of research which will assist in a better understanding of the disease and the method of action of insulin.

We have attempted to include in the program the results of basic work as well as reports of clinical studies with the sulfonylureas.

ALFRED FISCHER, M.D., (*Acting President, New York Diabetes Association*): The purpose of this Symposium is the exchange of information relating to the condition known as diabetes mellitus and its many related metabolic problems. Each year the amount of research done in this field is overwhelming and cannot be properly read and digested by more than a handful of its most knowing experimenters. We are fortunate that each year we are able to collect at one time, in one place, many of the most prominent workers in this field and thus are able to lay before you, in outline fashion, the results of much of what they are doing. The experiment which we started four years ago has now become an established fact, and we look forward each year to an Annual Day greater and more rewarding than that of the year before. The Symposium has become so successful that it has brought physicians and research workers throughout the country to it, and we have had to secure a larger auditorium in order to accommodate all those who wish to attend. This year's Symposium will again be published in DIABETES, *The Journal of the American Diabetes Association.*

It would have been difficult and almost impossible for us to have held the Symposium again this year without some financial aid. Each year some person or organization has come along and recognized our need. This year Eli Lilly and Company has undertaken to finance the greater part of the expense involved in arranging the Symposium. To this company we wish to express our great thanks.

I would like to remind those of you who are not members of the Clinical Society of the New York Diabetes Association that membership is open to all physicians, research workers, and teachers in the field of diabetes and its related subjects. The purpose of the Society is to encourage clinical and laboratory research in the field of diabetes, and to disseminate knowledge which may be helpful to diabetics everywhere. If you are interested in joining, write to the New York Diabetes Association, Inc., 104 East 40th St., New York 16, N. Y.

Presented at the Auditorium, Hunter College, 695 Park Ave., New York, N. Y., on Friday, Oct. 12, 1956.

Cell Types of the Islets of Langerhans and the Hormones They Produce

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The pancreatic islet tissue was first described by Paul Langerhans in 1869[1] while he was still a medical student. Langerhans identified a new cell type in the pancreas which had not heretofore been observed. He noted that these cells were grouped together in rounded cell masses measuring between 120 and 240 microns in diameter and that these were distributed throughout the parenchyma of the pancreas. Laguesse,[2] some twenty-five years later, named these structures the islets of Langerhans. Laguesse studied the cytology and development of the islets.[2, 3] He observed that the islet cells contained granules which could be seen in the living cell examined in serum and that these granules could be stained by safranine or gentian violet.

CELL TYPES PRESENT IN ISLET TISSUE

The existence of different cell types within the islet of Langerhans, although noted by earlier investigators,[4, 5, 6] was carefully studied by Lane[7] working in Prof. R. R. Bensley's laboratory. Lane studied the solubility of the granules within the islet cells of the guinea pig and found that whereas the granules of the alpha cells were precipitated by 70 per cent alcohol, they were dissolved following aqueous chrome sublimate fixation; by contrast the beta cell granules were precipitated by aqueous chrome sublimate fixative and dissolved by alcohol. Thus, depending upon the fixation employed, Lane was able to stain selectively either the alpha or the beta cells of the islet tissue using Bensley's neutral gentian stain. This neutral stain was prepared by mixing gentian violet (or crystal violet) with orange G in equimolar amounts and separating the precipitate thus formed. The granules of the alpha and beta cells could be clearly differentiated from each other and from the zymogen and prozymogen granules of the pancreatic acinar cells. In the guinea pig the beta cells, which constitute the majority of the cells within the islets, differ from the alpha cells with respect to their size and their nuclear morphology. In other species, however, although the size and nuclear differences between the alpha and beta cells are less clear, these two cell types can be readily differentiated by the selective staining of their granules.[8]

Bensley was able to stain simultaneously both the alpha and beta cells within a single preparation.[9] The pancreas was fixed in an osmic acid-bichromate mixture and stained with aniline acid fuchsin, methyl green; the granules of the alpha cells were stained red whereas those of the beta cells were stained lilac. Bensley also found a third cell type in the islet tissue of the guinea pig which he described as the "clear cell." This cell did not contain any specific granules. Since the nucleus of this third cell type resembled the nucleus of the alpha cells and since the number of granules in the alpha cells had been found to vary considerably from cell to cell, Bensley suggested that this third cell type might be a precursor of the alpha cell.[9]

Bloom in 1931 noted the existence of a fourth cell type in the islet tissue of the human pancreas[10] stained with the Mallory (Heidenhain) azan method. This cell type, however, has not been observed in all of the species studied.[8] Bloom believes that the blue staining delta cells,[10] as seen in the Mallory azan method, differ from the nongranular cells (C cells) which Bensley described in his aniline acid fuchsin, methyl green stained preparations.[9] In applying the Mallory azan method, the pancreas is initially overstained with azocarmine. Following a progressive decolorization, the sections are then counterstained with a mixture of aniline blue and orange G. The alpha cells are stained red, the beta cells are stained orange yellow, the delta cells are stained blue. Gomori has modified this stain somewhat[8] and he has found that the staining of the alpha cell granules could be intensified by a preliminary permanganate oxidation. Anomalous staining of the

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beta cell granules, however, has been observed in occasional blocks of human pancreatic tissue and in the guinea pig where the staining characteristics of the alpha and beta cells are just the reverse of those found in most other species studied.

Gomori has also used the chrom hematoxylin phloxine method to differentiate the cell types in the islet tissue.[11] Here, too, sections are oxidized with potassium permanganate prior to their staining. The beta cell granules are stained blue with the chrom hematoxylin; the alpha cells are stained red with the phloxine.

The aldehyde fuchsin method introduced by Gomori[12] in 1950 has been modified slightly by various investigators.[13, 14] A preliminary oxidation of the sections with an appropriately dilute acid permanganate solution, improves the staining of the beta cell granules. Although the aldehyde fuchsin method stains elastic fibers and other constituents as well, the stain has proved to be one of the most reliable procedures for demonstrating the beta cell granules. Bouin fixed material has given the best results.[12] The aldehyde fuchsin stain is prepared by ripening the basic fuchsin plus paraldehyde in an alcoholic hydrochloric acid solution; the aldehyde fuchsin is presumed to be an addition product of the aldehyde and the dye. Using this procedure the beta cell granules are stained a deep purple. The alpha cell granules are unstained. Because the cytoplasm of these cells is clear, it can be counterstained with a variety of dyes such as light green, orange G or phloxine.

Various silver staining methods have also been used to stain the islet tissue in the pancreas. Mankowski[6] in 1902 reported that the injection of silver nitrate resulted in the appearance of black specks within the islet tissue of the guinea pig; however, he found that the pancreatic acinar cells did not stain with silver. Van Campenhout[15] impregnated blocks of pancreatic tissue with silver nitrate and found that the granules of the pancreatic alpha cells as well as those of the intestinal enterochromaffin cells were stained with silver. Ferner, using the Gros Schultze method, found that the alpha cell granules were selectively stained with silver and he suggested that this method could be used for their positive identification.[16, 17] Donaldson and Humphrey, using Bodian's protargol method, were likewise able to stain certain of the cells in the islet tissue.[18] On the other hand, Masson using his own silver method, found that none of the islet cells stained with silver even though this procedure was adequate for the identification of argentaffin cells.[19] Negative results were likewise reported by Gomori[8] who used the Bielschowski-Foot silver impregnation method and by other investigators.[20, 21]

ISLET TISSUE AS A SOURCE OF ANTIDIABETOGENIC HORMONE

Although the islets of Langerhans were discovered in 1869,[1] more than twenty years elapsed between this time and the discovery by Minkowski that total pancreatectomy in the dog produced diabetes.[22] This new finding naturally stimulated research on the islet tissue. Laguesse, on the basis of his studies carried out in many vertebrate species,[3] suggested that the islet cells produced an internal secretion. This prediction which he made on purely anatomical grounds was based upon the finding of a rich blood supply in the islet tissue, upon the absence of lumina in the cell cords of the islets, and upon his inability to demonstrate connections between the islets and the ducts. Laguesse reasoned that since the islet cells did not connect with the ducts, the blood stream must provide the outlet for their secretion.

This thesis is supported by transplantation experiments[23] where it was shown that small portions of pancreas, detached from the bowel and transplanted under the skin, are sufficient to prevent glycosuria in the depancreatized dog. When the transplanted pancreatic graft remnants were removed the glycosuria was re-established. Because the elimination of the external secretion of the pancreas with the consequent impairment of digestion did not play a role in diabetes, it was postulated that the transplanted islet tissue prevented diabetes because it provided an internal secretion acting through the blood stream. The duct ligation experiments[24] likewise supported the thesis that islet tissue alone mediated the antiglycosuric function of the pancreas. For although duct ligation eliminated the outflow of the pancreatic juice and resulted in a degeneration of the acinar tissue, the secreting elements of the islets of Langerhans remained and glycosuria did not develop.

Many of the later studies on the status of the islets of Langerhans, following duct ligation of the pancreas, were contradictory. Therefore it is not surprising that many investigators challenged the concept that the islet tissue had an endocrine function. Dale, in particular, staunchly maintained that the islets represented the exhausted state of the pancreatic secretory acini and that the number of islets varied with the nutritional state of the animal and with the secretory activity of the pancreas.[25] Even Laguesse, who had supported the thesis of islet endocrine function, believed

that a reciprocal transformation between islet and acinar tissues could take place under certain circumstances.

Bensley's studies[9] in 1911 on the islets of Langerhans did much to clarify this state of confusion. Bensley developed supravital technics which permitted him to stain all of the islets in the pancreas and to stain the duct system independently in the same preparation. It was now possible to count the number of islets, to study the functional changes in the islets of Langerhans, to determine their relationship to the ducts, and to clarify the effects of duct ligation. Bensley's studies left little doubt that the islets of Langerhans were independent structures which had an endocrine function.

The isolation of insulin from the pancreas by Banting and Best[26] and the demonstration that insulin was effective in the treatment of diabetes clarified once and for all the antidiabetic function of the pancreas. Although this discovery did not settle the question of the site of insulin synthesis, a number of related experiments have been reported which support the thesis that the islet tissue is the source of insulin. For example, it had been shown previously that the number of islets in the tail of the pancreas was greater than that in the head.[9] When the insulin content per gram of pancreas was measured, the insulin content in the tail was likewise greater than that found in the head.[27] Large amounts of insulin could also be extracted from the duct ligated pancreas in which the acinar tissue had degenerated and in which only the ducts and islet tissue remained. In certain species of fish where the islet tissue is anatomically separated from the acinar pancreas,[28, 29] it is possible to determine the insulin content of the islet tissue. Using this material McLeod found that large amounts of insulin could be isolated from the islet tissue.[30]

BETA CELLS AS A SOURCE OF INSULIN

An early indication that the beta cells were the site of the antidiabetic hormone came from the work of Homans[31, 32] and Allen[33] some nine years prior to the discovery of insulin. These investigators studied the cytological changes in the islets of Langerhans following partial pancreatectomy and correlated these with the development of diabetes. They found that when more than 80 per cent of the pancreas was removed that there was a progressive appearance of manifest diabetes. Coinciding with the onset and development of diabetes, there was a progressive degranulation, hydropic degeneration and disappearance of the beta cells. The alpha cells in islet tissue did not show cytological change.

The finding in 1943[34, 35, 36] that the production of diabetes following alloxan injection could be correlated with the selective destruction of the beta cells whereas the alpha cells were not affected by alloxan, lends further support to the thesis that insulin is secreted by the beta cells.

The Toronto group[37, 38] have compared the insulin content of the pancreas of man, as measured by bioassay procedures, with the beta cell granule content as estimated cytologically using aldehyde fuchsin stained material. They found good correlation between the number of beta cell granules seen and the actual insulin content as determined by bioassay. Similar correlations have also been reported by Bell.[39] It is of interest to note that, in similar studies carried out earlier,[40] using other cytological staining methods (i. e., the Mallory azan, the chrom hematoxylin phloxine, or Bowie's modification of the neutral gentian method) there was poor correlation between the insulin content of the pancreas as measured by bioassay methods and the beta cell granule content as measured cytologically. Thus, the granules stained by these earlier staining procedures appear to be less specific than those stained by the more recently developed aldehyde fuchsin method. There is good evidence, therefore, that in the normal pancreas the beta cell granule, as stained by the aldehyde fuchsin method, represents stored insulin or a precursor of insulin. It should be noted, however, that insulin containing islet cell tumors may lack beta cell granulation.[41] Furthermore, although there is a statistically significant correlation between the insulin content and beta cell granule content in both the normal and in the diabetic human pancreas there are differences between these two groups. When a graph was prepared in which the beta cell granule content was plotted against the insulin content, it was found that the slope of the plot in the diabetic differed from that seen in the normal.[38] Therefore, one must not conclude that all of the insulin which is present in the beta cell is necessarily present as a microscopically visible secretion granule.

In considering the over-all problem of diabetes, insulin synthesis and the beta cell, it is important to keep in mind that there are a number of separable steps involved. The insulin molecule must first be synthesized from its constituent amino acids. The synthesized insulin can be secreted directly into the blood stream or it can be stored as a secretion granule in the beta cell. The insulin which is stored as a secretion granule must be released from the beta cell when it is needed. The insulin which is released must be able to reach the peripheral tissue without being destroyed. Finally, the

peripheral tissues must be able to respond to insulin. Diabetes could be the result of an absence of beta cells, a block in the insulin synthetic mechanism, an inability to release stored insulin, an increased rate of destruction of insulin or a lack of responsiveness of the target organs to insulin.

SYNTHESIS OF INSULIN BY BETA CELLS

Some of the steps which are involved in the synthesis of insulin are illustrated in figure 1. The number of the component amino acid residues found in the insulin molecule are indicated by the numbers in parentheses; those amino acids which are marked with an asterisk are essential and they must be supplied in the diet. The synthesis of insulin would depend among other things upon the presence of an adequate supply of these essential amino acids for they must be available to the beta cell for insulin synthesis. The sulfur-containing amino acids may be particularly important for insulin synthesis since these constitute 12 per cent of the amino acid residues in the insulin molecule.[42] These must be supplied in the diet either as cystine (or cysteine) or as methionine, which can be converted to cystine within the body. It has been found that the feeding of a diet low in the sulfur-containing amino acids resulted in a decreased insulin content in the pancreas.[44]

The component amino acids are joined together in a specific sequence by acid amide linkages to form two component polypeptide chains, the A and B chains which contain twenty-one and thirty amino acids respectively.[43] The arrangement of the amino acids within the polypeptide chain is not linear; the backbone of the chain is believed to be folded into a compact spiral structure in which the carboxyl group of a given amino-acid in the chain is adjacent to the amide group of an amino-acid four units along the chain (see figure 1). This would provide a basis for hydrogen bonding and the forces needed to hold the spiral together. The two component A and B chains, however, are held together by two interchain disulfide bridges; in addition there is a third intrachain disulfide bridge within the A chain.[43] In figure 2 the amino acids are shown entering the beta cell where they are incorporated into the insulin molecule. The primary insulin molecule synthesized has a molecular weight of 6,000 and it is illustrated in the figure as two cross-linked spiral structures. Practically nothing is known about the mechanism or the enzymes by which the beta cells synthesize insulin. Since the mitochondria are active metabolic sites within the cell they may well play a role in insulin synthesis by supplying the energy (such as ATA) needed for amino acid transformation and peptide bond synthesis. The actual site of insulin synthesis may well be within another portion of the cytoplasm, for in the liver cell, at least, protein synthesis can take place in other isolated cytoplasmic fractions of the cell, i.e., the microsome fraction.

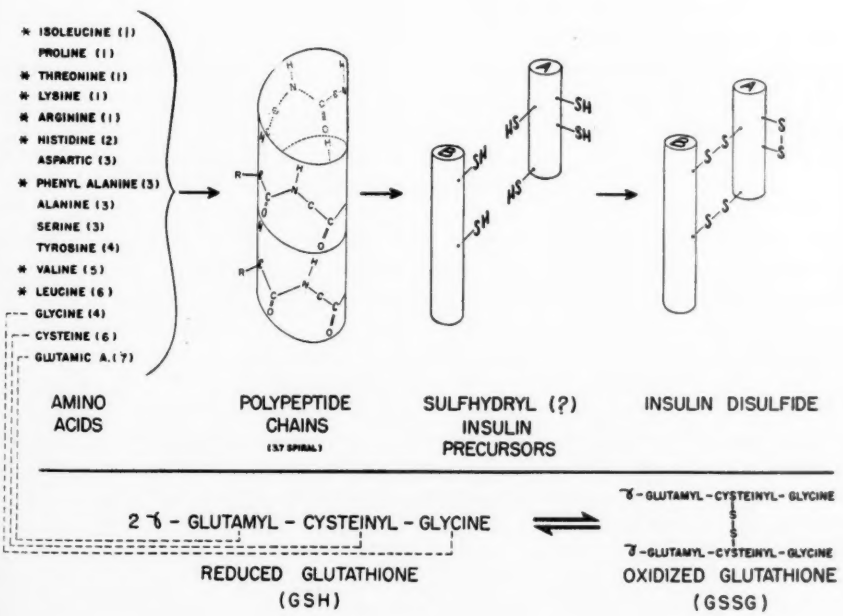

FIG. 1. Insulin synthesis.

It has been postulated on purely hypothetical grounds[45] that the component peptide chains of insulin might be synthesized separately, possibly as the sulfhydryl peptide chains, and that the two sulfhydryl peptide chains might be joined together enzymatically to form the insulin molecule by oxidizing the sulfhydryl groups to disulfide bonds (figure 1). This would be analogous to the formation of oxidized glutathione,[46] where two sulfhydryl containing tripeptide molecules are joined together enzymatically in a similar fashion by oxidizing their sulfhydryl groups with the formation of an interpeptide disulfide bridge. Nothing is known about the enzymes which synthesize the specific peptide chains of insulin or factors which join or fold the peptide chains into the highly specific insulin molecule. An interference in insulin synthesis could conceivably occur at any of the many stages in the process, and although a variety of factors have been shown to affect the insulin content of the pancreas,[27] we do not as yet know whether these factors influence insulin synthesis, insulin storage or insulin release from the beta cell.

STORAGE OF INSULIN AS A SECRETION GRANULE IN BETA CELLS

Although the newly synthesized insulin may be directly excreted into the blood stream, it may also be stored within the beta cell for later use as a secretion granule. The insulin which has been isolated from the pancreas by chemical means has a molecular weight of about 6,000,[47] and it is soluble at neutral *p*H.[48] The insulin, or insulin precursor, which is stored as, or in, the beta cell granule would appear to be insoluble at the *p*H of the cell. Granules which contain insulin and which are presumed to be secretion granules can be centrifuged out of fish islet tissue homogenates.[49, 50]

The component insulin molecules (M.W.=6,000) within the secretion granule are held together by an unknown factor which is represented in figure 2 by the black dots. The islet tissue contains large amounts of zinc[51] and it is of interest to note that the insulin which is isolated from the pancreas by standard biochemical procedures likewise contains some zinc.[52] It has been suggested that the insulin monomers of molecular weight equal to 6,000 may be cross-linked by zinc[53] and that the histidine residues in the B chains of insulin are joined together by metal binding with zinc.[54] The addition of zinc to insulin solutions at neutral *p*H and in the absence of phosphate ion, markedly decreases the solubility of insulin.[55] Although zinc may play a role in the aggregation of insulin, little is known about the precise nature of the ultra structure of the secretion granule or the way in which insulin is stored within the granule. If zinc were a component of the secretion granule, it might have to be selectively taken up from the blood stream by the beta cell. Although there may well be a relationship between zinc, insulin and the beta cell granule, there is no evidence to suggest that this is a specific relationship for it has likewise been shown that the alpha cells may also contain large amounts of zinc.[56]

RELEASE OF INSULIN FROM BETA CELLS

The beta cell releases its stored insulin into the pancreatic vein under the stimulus of hyperglycemia. When the glucose level of the blood perfusing the isolated pancreas is increased to hyperglycemic levels, the pancreas responds by putting out more insulin into the pancreatic vein.[57] Likewise, following the transitory elevation of the blood sugar which follows the injection of a single intraperitoneal dose of glucose, there is a transitory partial degranulation of the beta cells;[58] the number of beta granules returns to normal as the blood sugar is restored. Thus the release of the stored insulin from the beta cell must be associated with an active disaggregation of the beta granules and with a solubilization of the stored insulin. This solubilization might thus provide the basis for a rapid release of insulin into the blood stream. Although it is clear that the stimulus of hyperglycemia can bring about the release of insulin, the mechanism by which this release is accomplished is not known.

One must keep in mind that even though the pancreas may be able to synthesize and store insulin normally, a defect in the insulin release mechanism could produce diabetes. Thus, if a given diabetic subject were unable to release the insulin that is stored within the beta cell in response to the stimulus of hyperglycemia, then this diabetic subject might well have a "normal" insulin content in the pancreas even though the blood insulin levels (if we could measure them) might be decreased. On the other hand, with a partial impairment in the insulin release mechanism, the pancreas might be able to release insulin in response to a greater than usual hyperglycemic stimulus. In this instance if the blood sample were drawn from a diabetic subject at a time when the sugar level was markedly elevated then the blood insulin level might approach the "normal range," in spite of the fact that the pancreas might have a decreased capacity to release insulin into the blood stream (at a normal blood sugar level) in response to a physiologic hyperglycemic stimulus. Thus, if we are to differentiate adequately between the possible types of human diabetes, it will be important to be able to measure the capacity of the pan-

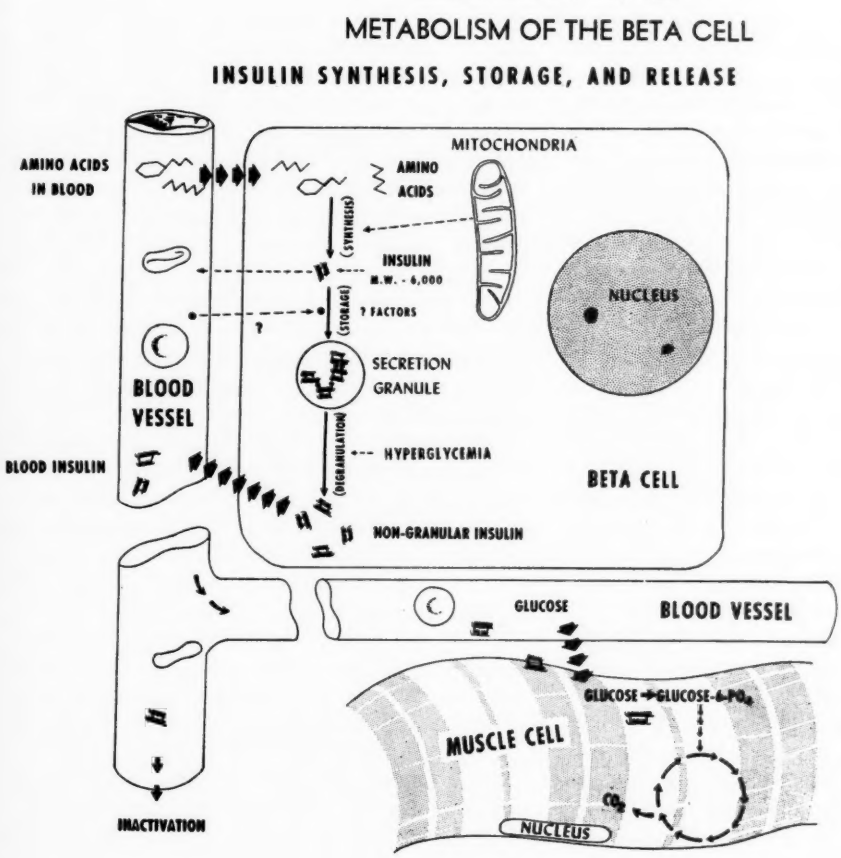

FIGURE 2.

creas to secrete insulin into the blood stream with reference to the absolute blood sugar level at the time the blood sample was taken, and in response to controlled levels of induced hyperglycemia. The absolute insulin contents of the blood or pancreas may not be indicative of the ability of the pancreas to secrete insulin in response to the usually physiologic stimuli.

ALPHA CELLS AS A SOURCE OF GLUCAGON

In considering the functional significance of the two cell types in the islets of Langerhans, it is of interest to recall the statement made by the late Prof. R. R. Bensley. In 1915 Bensley wrote,[59] "Interest has centered around the phenomena of diabetes, and workers have sought by various experimental methods to show that the work done by the pancreas in connection with the control of carbohydrate metabolism was the function in particular of the islets of Langerhans. That this is a narrow point of view whether we regard it from the standpoint of the derangements of metabolism in diabetes or from the standpoint of the internal secretory activity of the pancreas, is obvious, for the fact that the islets are themselves composed of two independent sorts of internal secretory cells would be sufficient to suggest that their internal secretory function is twofold, and many studies indicate that there is more to diabetes than a deficient utilization of carbohydrate material."

Murlin, et al.[60] in 1923, noted the presence of an insulin contaminant which he called glucagon and which upon intravenous administration produced an initial transitory elevation in the blood sugar preceding the hypoglycemia which characteristically follows insulin administration. Glucagon was later isolated from the pancreas and purified independently by two groups of investigators.[61, 62] Glucagon increases the blood sugar presumably because it activates the liver phosphorylase system and thereby increases the glycogen breakdown in the liver.[63]

Sutherland and De Duve[64] have suggested that islet

tissue was the source of glucagon. Following duct ligation they were able to extract increased amounts of glucagon from the pancreatic remnant in which only the islet tissue and the ducts remained. Similarly, they reported that the glucagon content in the tail of the pancreas was ten times greater than that found in the head. Thus, the amount of glucagon extractable from the pancreas parallels the islet tissue content.

Several studies have implicated the alpha cells as the most probable source of glucagon. The amount of glucagon which can be extracted from the pancreas was not decreased following alloxan destruction of the beta cells;[64] similarly, glucagon could be extracted from the duct ligated, alloxan injected pancreas.[65] Thus, the destruction of both the acinar and the beta cells did not decrease the glucagon content of the pancreas. In the dog, the alpha cell content of the islet tissue varies in the different portions of the pancreas.[66] For example, the uncinate process contains islets that are practically devoid of alpha cells whereas the tail of the pancreas contains islets with the usual number of both alpha and beta cells. On the basis of semiquantitative estimates of the glucagon content of the dog pancreas, it has been suggested that the uncinate process appears to be devoid of both glucagon and alpha cells.[66]

The concept that the alpha cells are the site of glucagon synthesis is complicated by the finding that a chemical constituent with physiologic properties similar to glucagon has also been isolated from the gastrointestinal tract of some species.[64] In explanation of this apparent contradiction, Sutherland and De Duve[64] have suggested that the argentaffin cells of the gastrointestinal tract may likewise be a second site of glucagon synthesis. This suggestion was made because of the reported finding that both the alpha cells in the pancreas and the argentaffin cells in the intestinal tract can be stained by certain of the silver stains.[15, 16, 17] Other investigators, however, have denied that there is any relationship between the pancreatic alpha cells and the silver staining argentaffin cells in the intestinal tract because the latter can be stained by methods which do not stain alpha cells.[8, 19, 20]

The finding that the injection of cobalt[67] or Synthalin[68] produced a selective destruction of the alpha cells in the islet tissue suggested that these compounds would be useful in elucidating the cellular source of glucagon synthesis. However, it has been shown that whereas these compounds produce hydropic degeneration and degranulation of alpha cells, they do not produce complete disappearance of the alpha cells.[69] Although some investigators have reported that the glucagon content of the pancreas is decreased following cobalt[70, 71] and Synthalin[72] treatment, others[73, 74, 75, 76] have not found any change in the glucagon content following the administration of cobalt.

Since cellular atrophy has frequently been observed to follow the injection of large amounts of the hormone produced by the given cell, it is of interest to note that one investigator has reported that the administration of glucagon causes an atrophy of the alpha cell.[77] However, similar atrophy has not been reported in other studies where large doses of glucagon were administered.[78]

A number of investigators have attempted to study the metabolic alteration produced in both normal and diabetic animals following cobalt administration and destruction of the alpha cells.[70–76] These experiments are complicated in their interpretation because part of the hyperglycemia which follows cobalt administration is clearly extrapancreatic in origin, as is indicated by the following observations. Cobalt injection produces hyperglycemia in the depancreatized dog which is devoid of the pancreatic alpha cells.[71] Cobalt injection failed to elicit a hyperglycemic response in animals previously treated with certain sympathetic blocking agents.[79] The administration of cobalt plus methionine produced hyperglycemia in the absence of demonstrable alpha cell damage.[80]

It has also been claimed that the alpha cell damage which follows Synthalin administration is secondary.[81] Similar secondary alpha cell degeneration has been reported to follow the liver damage induced by phosphorus, carbon tetrachloride, chloroform and ethionine.[81]

Although it is probable that the pancreatic alpha cells are the site of glucagon synthesis, there are unfortunately no available methods for the positive cytochemical identification of glucagon within the alpha cell. The glucagon which is synthesized can presumably be stored as a secretion granule within the cell. One can further postulate that the glucagon which is stored as a secretion granule may be disaggregated and released into the blood stream as needed. In the case of insulin, it has been shown that although many staining methods can be used for the identification of the beta cells, there was good correlation between the insulin content of the pancreas and the beta granule content only when the aldehyde fuchsin staining method was used. Thus in the case of glucagon, although many of the cytological staining methods can be used for the identification of the alpha cells, there is no evidence to suggest that any of the staining methods devised thus far are adequate for the cytological demonstration of stored glucagon.

Until an adequate cytochemical method is available, one might expect that any correlated study of the cytological and functional states would be complicated and difficult to interpret.

The glucagon which is stored in the cell could likewise exist in both a granular and a nongranular form. If the alpha cells were depleted of stored glucagon, the cytoplasm of these cells might not show any change if the cells were stained by a nonspecific staining procedure. Thus the failure to find changes in alpha cells following massive glucagon administration could be the result of inadequate staining methods.

It would seem doubtful whether the silver staining reaction of the granules in the alpha cells, or of the extrapancreatic cells, represents a specific reaction for glucagon. In general the silver staining methods are precarious and lack specificity. Since the alpha cells can be stained by some but not by other of the silver methods and since certain of the silver methods do stain the intestinal argentaffin cells but not pancreatic alpha cells, there is good reason to doubt that the silver reaction is a specific one for either glucagon or glucagon secreting cells.

Thus, the possible existence of an extrapancreatic glucagon secreting cell must be clarified by a more positive cytochemical identification of the hyperglycemic factor in the extrapancreatic sites and by the positive chemical identification of the hyperglycemic glucagonolytic factor which has been isolated from the gastrointestinal tract.[64] This identification should be possible now that the pancreatic glucagon has been crystallized[82] and its amino acid sequence determined.[83] If it can be shown that there is an extrapancreatic site of glucagon synthesis, this finding would obviously complicate the interpretation of many of the reported experiments for pancreatectomy would not eliminate all of the glucagon secreting cells. Likewise, it would be absolutely essential to evaluate the cytological changes in the extrapancreatic as well as the pancreatic glucagon secreting cells.

The functional and biological significance of glucagon will be discussed by others in this Symposium. However, it is clear that the complete story of the function of glucagon as well as the precise role that glucagon plays in diabetes is still not clear.

DELTA CELLS OF THE PANCREATIC ISLETS

These cells which have been described in the islet tissue of some species more than twenty-five years ago have been largely ignored. Although the delta cells are present in the human pancreas,[10] they are not found in all species studied.[8] But, though the delta cells have not been seriously considered as a source of glucagon, it should be pointed out that most of the evidence which has been cited in support of the alpha cell origin of glucagon is based primarily on the exclusion of both the acinar and beta cells as sources of glucagon. Histologists, primarily because of their training, usually have an unquestioning faith in the cytologically differentiated structures which they see under the microscope. The failure to demonstrate physiologic or biochemical significance for these structures usually does not decrease the histologist's belief in the importance of these structures. Experience has taught us that it is not possible to demonstrate the effect of a hormone which acts on an enzyme or an intermediary metabolic step that has not yet been discovered and that many decades may elapse between the time a cell type is discovered and its effect elucidated. The pancreatic delta cells may some day be shown to have an important endocrine function. Therefore, in thinking about the role of the pancreatic islet tissue, we must include the possibility that there may be one or more additional hormonal factors which play a physiologic and metabolic role.

SUMMARIO IN INTERLINGUA

Le Typos de Cellulas in le Insulas de Langerhans e le Hormones que Illos Produce

Le typos de cellulas trovate in le tessuto del insulas de Langerhans es discutite con referentia special al varie technicas de coloration usate in lor identification positive. Le specificitate del varie chromoreactiones usate in differentiar le cellulas alpha e beta es evalutate criticamente. Es presentate e discutite le argumentos in favor del theses que (1) le tessuto insular es un fonte del hormon antidiabetogene e (2) le cellulas beta es le fonte de insulina. Es summarisate nostre cognoscentias currente in re le synthese de insulina per le cellulas beta, le immagasinage de insulina como granulo de secretion intra le cellula beta, e le liberation de insulina ab le cellula beta. In considerar le etiologia de diabete le autor judica que iste morbo pote esser le resultato de (a) un absentia de cellulas beta, (b) un bloco in le mechanismo del synthese de insulina, (c) le incapacitate de liberar insulina immagasinate, (d) un accelerate destruction de insulina, e (e) un manco de responsa a insulina del parte del organos peripheric. Es signalate in plus que mesmo si nos poteva determinar le quantitate de insulina que es continite in le sanguine o in le pancreas, le valores obtenite non reflecterea necessarimente le capacitate del pancreas de secerner insulina a in le fluxo sanguinee in responsa al stimulos physiologic.

Es presentate e evalutate criticamente le datos que pote esser citate in supporto del conclusion que le cellulas alpha es un fonte de glucagon. Es signalate que le correlation inter le cytologia del cellulas alpha e le interpretationes physiologic del effectos de agentes a cytotoxicitate pro cellulas alpha es rendite plus complicate per le absentia de un methodo specific que pote esser usate pro le positive identification histochimic de glucagon intra le cellula. Le natura e le rolo possibile del cellulas delta es etiam prendite in consideration.

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DISCUSSION

BRUNO W. VOLK, M.D., (*Brooklyn*): I wish to congratulate Dr. Lazarow on his very beautiful and detailed presentation. As he just mentioned, in studying the function of the normal pancreatic islets, it is important to obtain a clear picture of their morphologic characteristics. Being a pathologist, I feel compelled to show a few additional slides of recent work. These were prepared in conjunction with my associate, Dr. Sydney S. Lazarus.

The first slide demonstrates a trichrome stain of the pancreatic islet of a normal rabbit. The alpha cells are purple, the beta cells are pink and the D cells are clear blue. As Dr. Lazarow pointed out, the identification of the beta cells as the source of insulin was based originally, among others, on the observation that they become degranulated in partially depancreatized animals and by the injection of anterior pituitary extract. More recently it has been shown that degranulation of the pancreatic beta cells occurs in animals with cortisone diabetes.

The next slide demonstrates an aldehyde fuchsin stain of a normal rabbit pancreas. The beta cell granules are distinctly blue and show polarization around the blood vessels.

The next slide demonstrates the pancreatic islet of a rabbit that has been extensively treated with cortisone. There is almost complete degranulation of the beta cells with only a few cells left containing granules.

In the degranulated beta cells the mitochrondria can be clearly identified morphologically by special staining procedures indicating that they are separate structures distinct from the beta cell granules. This fact is in conflict with the recent claim of Maske that insulin and the beta cell granules are morphologically related.

The ultimate proof that insulin originates from the beta cells was the discovery that alloxan selectively destroys the beta cells and thereby produces diabetes. The next slide demonstrates Gomori's chrom alum hematoxylin phloxine stain of the pancreas of an alloxan diabetic rabbit. There is necrosis of the beta cells which are surrounded by a rim of intact alpha cells.

Most individuals who have studied the pancreatic islets have used the Gomori procedure. However, this staining technic is not entirely satisfactory since the phloxin is used as a general cytoplasmic stain and does not completely differentiate all cellular elements, including the D cells or ductular cells which may enter the islets.

The next slide demonstrates a chrom hematoxylin Ponceau fuchsin stain of a normal pancreatic islet which is preferable for identification of the islet cells. The alpha cells are red, the beta cells are blue and the D cells are clear.

The next slide shows a pancreatic islet stained by the same procedure of a rabbit treated with cortisone. There is degranulation of the beta cells as well as ductular proliferation into the islets noticeable. The epithelium of the duct is unstained. In many morphologic experiments in which unspecific staining methods have been used, this proliferating ductular epithelium has been confused with the beta cells. This slide also demonstrates that planimetric measurements which have been used to identify the size of the islets may be open to considerable error.

A few years ago, Dr. Lazarus, Dr. Goldner and myself were studying the relationship between glucagon and the alpha cells. Using cobalt as a method to destroy the alpha cells we observed that pancreatic extracts assayed qualitatively from animals who had received cobalt, contained as much glucagon as those of untreated animals. This led us to the conclusion that glucagon was probably not derived from the alpha cells.

More recently, Bencosme and Lazarus, in studying pancreatic extracts of cobalt-treated guinea pigs and dogs determined that in order for a qualitative method of assay to show reduction of the glucagon content that there must be an almost 100 per cent absence of the alpha cells. This and other investigators' work then suggested the possibility that the alpha cells do produce glucagon.

However, there is other conflicting evidence. It is well known that the hyperglycemic principle can be extracted from the gastric mucosa of the dog, originating supposedly from the silver positive cells. However, these cells have been shown to be tinctorially not identical with the alpha cells.

It has recently been claimed that IPTD and other hypoglycemia producing sulfonylurea derivatives lower the blood sugar by virtue of their alphacytotoxic action, thereby eliminating the source of glucagon. Subsequently it has been shown, however, that these sulfa drugs neither damage the alpha cells nor that glucagon is involved in their effect on the blood sugar.

I feel then that although the inferential evidence is suggestive that glucagon is derived from the alpha cells, the matter at the present appears still somewhat confused and requires further clarification.

As to the third cell of the pancreatic islets, the D cell has no known function and there is still some con-

troversy whether it is granulated. Its endocrine function is highly questionable.

GENERAL DISCUSSION

DR. HART (*Kew Gardens, New York*): I noticed from here that the D cells contained no nuclei.

DR. LAZAROW: No. They do have nuclei.

DR. JOHNSON: I would like to ask if there is any supported theory of the fact that there is a regeneration in these beta cells during the period of, say, convalescence in which there is a reduction in the blood sugar.

DR. LAZAROW: The problem which you raise concerning the regeneration of the beta cell is complex. There is evidence from animal studies that beta cell regeneration can take place under certain circumstances. When pituitary extracts have been administered to dogs for periods of fourteen to twenty-one days, the beta cells become irreversibly damaged and permanent diabetes is produced even though the administration of the pituitary extract is discontinued. If, however, during the period of pituitary extract administration, the induced hyperglycemia is controlled by the simultaneous administration of insulin, the beta cell damage is prevented and the dogs do not develop permanent diabetes. The problem of reversible damage to the beta cell is very important and it should be investigated further.

DR. LOEWI: Have you ever tried to centrifuge this insulin?

DR. LAZAROW: Dr. Loewi has raised a very interesting question. If the insulin is stored as a secretion granule within the beta cell it should be possible to centrifuge these granules. A number of years ago, when we started to work with the toadfish islet tissue, we prepared homogenates and we separated some of the particulate components from islet tissue. At that time we showed that the centrifuged particulate components would produce hypoglycemia when they were injected into mice. However, the quantitative determination of the insulin which was present in the small amounts of the various islet tissue fractions obtained posed a difficult problem. In order better to approach this problem, we have devised an antigen antibody method which might be useful for the quantitative identification of insulin in these various cell fractions.

Maske has recently fractioned pooled islet tissue obtained from fish and he has carried out insulin assays on the granules obtained. Insulin was recovered from the granular fraction and Maske has suggested that the insulin was present in the mitochondria. Cytological studies of the pancreatic islet tissues, and especially those carried out with the electron microscope, clearly indicate that the mitochondria and the beta cell granules are separate and distinct structures. I believe that it will be possible to separate these two components by differential centrifugation. This is an interesting area for research which we are presently pursuing.

DR. LOEWI: I want to know whether the hypoglycemia is a stimulant for insulin, whether it acts on the whole cell.

DR. LAZAROW: I wish that I could answer your question. The mechanism of the disaggregation of the secretion granules with the consequent liberation of insulin into the blood stream and the mechanism by which hyperglycemia effects this process is probably one of the most fundamental unanswered questions with regard to the physiology of the beta cell. I would be grateful for any suggestions that you might have concerning methods of approaching this problem.

Knowledge of Feeding Behavior

Within the past fifteen years the nature of the regulation of feeding has become clearer, with the result that our present understanding of the subject compares favorably, for example, with knowledge of the regulation of pulmonary ventilation. The two regulations appear to have many features in common. In the one case, respiration, the location of medullary "centers" has been established, while some of the factors affecting these centers have been identified, including the composition of the blood and reflexes from lungs and from the carotid and aortic bodies. In the other case, feeding, the important "centers" appear to lie within the hypothalamus. Reflexes from the digestive tract appear to be analogous to those from lungs and chemoceptors, while other reflex patterns and the composition of body fluids likewise participate in the regulation.

From the book *Modern Nutrition in Health and Disease* edited by Michael G. Wohl, M.D., and Robert S. Goodhart, M.D. Philadelphia, Lea & Febiger, 1955. Chapter "Physiology of Hunger, Appetite and Satiety" by John R. Brobeck, M.D., pp. 90-91.

The Amino Acid Sequence of Glucagon

W. W. Bromer, Ph.D., L. G. Sinn, A.B., A. Staub, Ph.D., and Otto K. Behrens, Ph.D., Indianapolis

INTRODUCTION

In 1953 Staub, Sinn, and Behrens[1] announced the preparation of crystalline glucagon, thus making available a small protein ideally suited for fundamental chemical and biological studies. As a part of these studies, research was initiated on structural aspects of the protein molecule. This report comprises a brief summary[2] of the work leading to the elucidation of the complete amino acid sequence of glucagon.

It is pertinent to consider briefly some of the reasons that such structural studies were attempted. Perhaps the principal motive behind protein structure research is not merely to reveal the amino acid sequence of the protein, but more important, to lay the foundation for understanding the biological activity of the protein on a molecular basis. For example, it might be asked what specific arrangement of amino acids or what chemical groupings are necessary in the glucagon molecule to elicit such a marked hyperglycemic response? Does a smaller portion of the glucagon chain retain activity? Why does glucagon have this effect, while insulin has another, and many other proteins have no known biological activity at all? Determination of the amino acid sequence is an important step leading to the understanding of the fundamental relationships between protein structure and activity.

EXPERIMENTAL

An important prerequisite to structural analysis is the determination of the amino acid composition of the protein. This was accomplished by hydrolyzing the protein in acid and analyzing the hydrolysate by the Dowex 50 chromatographic method of Moore and Stein[3] and the dinitrophenylation technic of Levy.[4] Table 1 depicts the results of these analyses with glucagon, and for sake of comparison, the amino acids in insulin.

It can readily be seen that the amino acid composition of glucagon is quite different from that of insulin. Methionine and trypotophan, for example, are present in glucagon but not in insulin; proline, cystine, and isoleucine are found in insulin but not in glucagon. The absence of cystine in glucagon is particularly significant because without cystine it is not possible for glucagon to contain the type of interchain bonding which holds together the two chains of the insulin molecule. This is one indication that the glucagon molecule is a single chain of amino acid residues. Also of particular interest are the single residues of lysine, alanine, histidine, valine, glycine, methionine, and tryptophan. These single amino acids provided points of reference by which the degradation products of glucagon were assigned positions in the chain. The twenty-nine amino acid residues in glucagon provide evidence for a minimal molecular weight of 3,485, which is in good agreement with physical studies.

As another preliminary to the actual sequential analysis it was important to determine the amino acids on the ends of the protein chain or chains. This informaiton provides evidence for the nature of the protein molecule, i.e., whether it is composed of single, cyclic,

TABLE 1

Amino acid composition of glucagon and insulin

Amino acid	Number of amino acid residues: Glucagon	Beef Insulin*
Aspartic acid	4	3
Threonine	3	1
Serine	4	3
Glutamic acid	3	7
Glycine	1	4
Alanine	1	3
Valine	1	5
Leucine	2	6
Tyrosine	2	4
Phenylalanine	2	3
Histidine	1	2
Lysine	1	1
Arginine	2	1
Methionine	1	
Trytophan	1	
Proline		1
Cystine		3
Isoleucine		1
Totals	29	48

*Harfenist, E. J., and Craig, L. C.; J. Am. Chem. Soc. 75:5528, 1953.

Presented at the Symposium on Insulin, Glucagon and the Oral Hypoglycemic Sulfonylureas sponsored by The Clinical Society of the New York Diabetes Association, Inc., on Oct. 12, 1956.

From the Lilly Research Laboratories.

branched, or multiple chains, as well as giving additional points of reference that are important in locating degradation products of glucagon. This type of determination is called end-group analysis. A convention of protein chemistry requires that the left hand end of the chain, when written, bears the free *a*-amino group and is called the amino- or N-terminal end; the right hand terminus containing the free α-carboxyl group is designated the carboxyl- or C-terminal end.

In the case of glucagon, the amino acid on the N-terminal end of the chain was determined by the Levy modification[4] of the classic dinitrophenylation method introduced by Sanger.[5] Histidine was determined to be the N-terminal residue of glucagon. One mole of Di-DNP-histidine was found per 3,500 gm. of the protein, giving further evidence that glucagon is a single chain of amino acids. Two methods of C-terminal analysis were employed, a chemical method called hydrazinolysis, and an enzymatic method using carboxypeptidase. In the chemical method, glucagon was reacted with hydrazine, forming hydrazides of all amino acids except the C-terminal one, which was found to be threonine. Carboxypeptidase is an enzyme which splits off one amino acid at a time from the carboxyl-end of the chain. Careful time studies with this enzyme confirmed the fact that threonine was the C-terminal amino acid of glucagon, and in addition, aspartic acid, asparagine, methionine, leucine, tryptophan, glutamine, valine, phenylalanine, and alanine were liberated. Since several of these amino acids occur only once in glucagon, these data were extremely useful later in orienting degradation fragments near the C-terminal end of the glucagon chain. The following is a summary of the evidence obtained thus far through amino acid analysis and end group determination:

His(ser_4, glu, gly, thr_2, phe, asp_2, tyr_2, lys, leu, arg_2)
—ala(glu_2, asp_2, phe, val, try, leu, met) thr[6, 7]

All available evidence clearly indicates that glucagon is a single chain of twenty-nine amino acids, containing histidine and threonine as the N- and C-terminal residues respectively.

The usual method of determination of the structure of an organic compound is based on the degradation of the molecule into smaller fragments, the identification of the pieces, and the integration of the degradation products in an unequivocal manner. The approach used in this problem has been to split the protein chain into fragments, called peptides, by the action of proteolytic enzymes. Chymotrypsin, trypsin, and subtilisin were the enzymes used in this study. The fragments of the glucagon chain formed by each of the enzymes were separated primarily by Dowex 50-X2 column chromatography, and these peptides were characterized by amino acid analysis, end group analysis, and in some cases further degradation with acid and carboxypeptidase. Considerable evidence has appeared in the literature showing that proteolytic enzymes, particularly trypsin, require rather specific substrates for hydrolysis. This characteristic of the enzymes is, of course, an advantage since selective splits are generally obtained, giving distinct degradation products amenable to isolation and characterization. Because of the possibility of enzyme-catalyzed rearrangements, however, it is necessary to study the degradation products formed from at least two different enzymes.

Subtilisin split glucagon into eleven peptide fragments, which were fractionated by chromatography on Dowex 50-X2 resin as illustrated by the effluent curve shown in figure 1. Each ninhydrin-positive peak, with the exception of S-8, was found to represent a pure, unique peptide fragment. The material from peak S-8 was readily separated into two homogeneous components by countercurrent distribution. The analysis and recovery of these peptides are presented in table 2. Generally, 50

TABLE 2

Peptides from the subtilisin digestion of glucagon

Peptide number		Peptide composition and analysis	Yield per cent
S-1		thr.ser	
	moles	.8 1.2	55
S-2		asp.thr	
	moles	1.1 1.0	80
S-3		asp(tyr,ser)	
	moles	1.1 1.0 1.1	50
S-4		asp.phe	
	moles	1.1 1.0	70
S-5		leu.met	
	moles	1.1 .9	60
S-6		gly(thr,phe)	
	moles	1.1 .9 1.0	85
S-7		leu(asp,ser,arg)	
	moles	1.0 .9 1.0 1.0	80
S-8A		his(ser,glu)	
	moles	.8 1.0 1.3	25
S-8B		val(glu,try)	
	moles	1.0 1.0 1.0	15
S-9		arg(ala,glu)	
	moles	1.2 .9 1.0	60
S-10		lys.tyr	
	moles	1.0 1.0	75

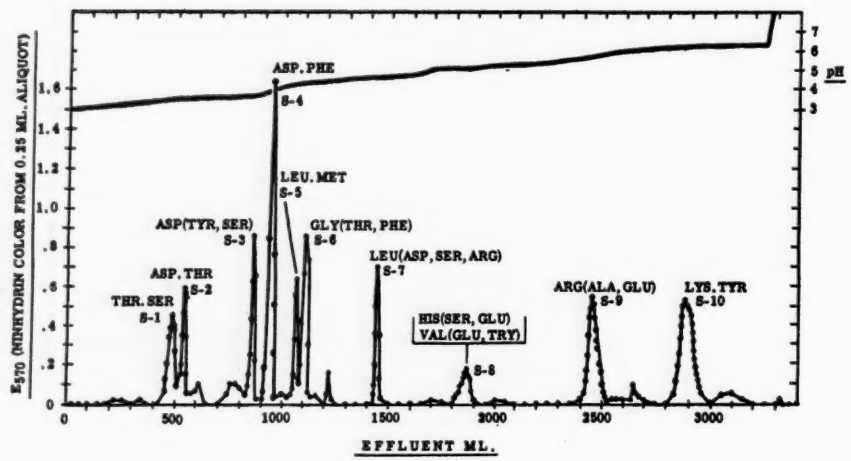

FIG. 1. Separation of the degradation products resulting from the 24 hr. subtilisin digestion of 176.2 mg. of glucagon. Chromatography was performed on a 1.5 x 50 cm. column of Dowex 50-X2 resin.

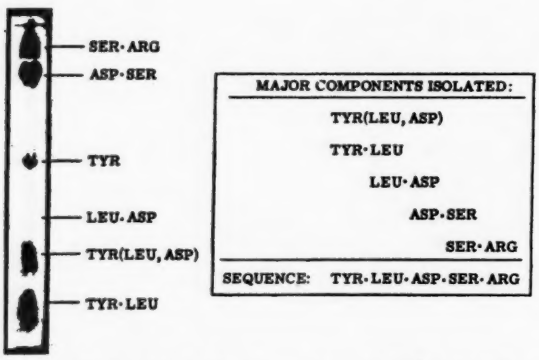

FIG. 2. Photograph of the paper chromatographic separation of the acid hydrolysis products of peptide LT-4, Tyr (leu, asp, ser, arg).

TABLE 3

Peptides from the 2¼ hour tryptic hydrolysis of glucagon

Peptide number		Peptide composition and analysis	Yield per cent
ST-1		tyr(leu,asp,ser,arg)	
	moles	1.0 1.0 1.0 1.0 1.0	70
ST-2		arg	20
ST-3		his(ser$_3$,glu,gly,thr$_2$,phe,asp,-	
	moles	.8 3.2 1.2 1.0 2.1 1.0 1.0 tyr,lys) 1.0 1.1	30
ST-PA		arg(ala,glu$_2$,asp$_2$,phe,val,try,leu,-	
	moles	1.0 1.1 2.0 1.8 .9 1.1 1.0 .9 met,thr) .7 1.2	50
ST-PB		ala(glu$_2$,asp,phe,val,try,leu,-	
	moles	1.0 2.1 2.1 .7 1.0 1.0 1.3 met,thr) .5 1.3	25

TABLE 4

Peptides from the chymotryptic digestion of glucagon

Peptide number		Peptide composition and analysis	Yield per cent
C-1		leu(met,asp,thr)	
	moles	1.0 .8 1.2 1.0	40
C-2		thr(ser,asp,tyr)	
	moles	1.0 1.0 1.1 1.0	65
C-3		val(glu,try)	
	moles	1.3 .6 1.0	60
C-4		his(ser,glu,gly,thr,phe)	
	moles	.8 1.0 1.1 1.1 1.1 1.0	50
C-5		leu(asp$_2$,ser,arg$_2$,ala,glu,phe)	
	moles	.9 1.9 1.0 1.8 1.0 1.0 .8	50
C-6		ser(lys,tyr)	
	moles	.9 1.3 1.0	50

per cent or greater yields of the fragments were obtained, and more important, the sum of the amino acids of the various peptides is identical in every respect to the amino acid composition of glucagon, indicating recovery of a reasonable quantity of all the components of the ruptured chain.

Similar fractionation of the enzymatic digests from the tryptic and chymotryptic splitting of glucagon resulted in the isolation of additional sets of peptide fragments, each set accounting for the entire glucagon chain. The data in table 3 show the fragments of glucagon formed by the action of trypsin after a 2¼ hr. digestion. The sum of the amino acid residues in the three major fragments, ST-3, ST-1, and ST-PA, again is identical to the composition of glucagon. It can readily be deduced from

TABLE 5

Peptide number	Hydrolytic agent	Structure of glucagon
ST-3	Trypsin (2¼ hrs.)	His(ser,glu,gly,thr,phe,thr,ser,asp,tyr,ser,lys)
S-8A	Subtilisin	His(ser,glu)
C-4	Chymotrypsin	NH_2
LT-5A	Trypsin (50 hrs.)	His·ser·glu·gly·thr·phe
S-6	Subtilisin	gly(thr,phe)
LT-3	Trypsin (50 hrs.)	thr·ser·asp·tyr·ser·lys
S-1	Subtilisin	thr·ser
C-2	Chymotrypsin	thr(ser,asp,tyr)
S-3	Subtilisin	asp(tyr,ser)
C-6	Chymotrypsin	ser(lys,tyr)
S-10	Subtilisin	lys·tyr
ST-1 LT-4	Trypsin (2¼ hrs. and 50 hrs.)	tyr·leu·asp·ser·arg
S-7	Subtilisin	leu(asp,ser,arg)
C-5	Chymotrypsin	leu(asp,ser,arg,arg,ala,glu,asp,phe)
ST-2 LT-5B	Trypsin (2¼ hrs. and 50 hrs.)	arg
S-9	Subtilisin	NH_2 arg(ala,glu)
ST-P	Trypsin	ala(glu,asp,phe,val,glu,try,leu,met,asp,thr)
LT-2	Trypsin (50 hrs.)	ala(glu,asp,phe,val,glu,try)
S-4	Subtilisin	asp·phe
C-3 S-8B	Chymotrypsin Subtilisin	NH_2 val·glu·try
S-5	Subtilisin	leu-met
C-1 LT-1	Chymotrypsin Trypsin (50 hrs.)	leu(met,asp,thr)
S-2	Subtilisin	NH_2 asp·thr
Amino acids from carboxypeptidase		NH_2 NH_2 NH_2 ala(glu,asp,phe,val,glu,try,leu,met,asp)thr
SUMMARY		NH_2 NH_2 NH_2 NH_2 His·ser·glu·gly·thr·phe·thr·ser·asp·tyr·ser·lys·tyr·leu·asp·ser·arg·arg·ala·glu·asp·phe·val·glu·try·leu·met·asp·thr

the unique amino acid composition that fragments ST-2 and ST-PB were formed from ST-PA. After prolonged incubation with trypsin two additional splits occur that cannot be discussed because of time limitations. The products of the chymotryptic digestion of the glucagon are presented in table 4. Again approximately 50 per cent or greater recovery of all the pieces of the glucagon chain was obtained.

All of the peptide fragments logically fit into the glucagon chain, indicating clearly that the molecule is a straight chain of twenty-nine amino acids. The evidence obtained thus far may be summarized in a partial structure as follows:

His(ser,glu)gly(thr,phe)thr.ser.asp.tyr.ser.lys.tyr.
leu(asp,ser,arg)arg.ala.glu.asp.phe.val.(glu,try)
leu.met.asp.thr

From the integration of the data it is apparent that many of the amino acid sequences can be written in only one manner; however all of the links have not been put into place, and certain peptides were selected for further degradation by acid or enzymes. In figure 2 is presented a photograph of the paper chromatographic separation of the acid-hydrolysis products of peptide LT-4, Tyr (leu, asp, ser, arg). From the composition of the degradation products the sequence of the amino acids in the parent peptide may be deduced. In this fashion, using either acid or carboxypeptidase treatment, the remaining unknown links were determined.

With the location of the four amide groups by chemical examination of selected peptide fragments, it was possible to formulate the entire amino acid sequence of glucagon. A summary of the evidence is presented in table 5.

DISCUSSION

Now that the amino acid sequence of glucagon has been revealed, it may be well to consider briefly the implications of this research, As is well known, many commercial insulin preparations exhibit a small transitory hyperglycemic effect when injected into humans or animals. It was considered that this hyperglycemic activity might be inherent in the insulin molecule, or, if different from insulin, it was at least very similar in chemical and physical properties to insulin. After the preparation of crystalline glucagon and the determination of its structure, there is no doubt that chemically the two proteins are quite different. It would not have been surprising to find a similarity in certain sequences or structures. Only recently it has been shown that intermedin and ACTH contain an identical heptapeptide sequence. In addition it is known that insulin, vasopressin, and oxytocin all contain a cyclic hexapeptide structure. Comparison of insulin and glucagon structures reveals no striking similarities. Only four dipeptide sequences are identical.

It is only through structural studies that these comparisons can be made. The synthesis of glucagon and the study of the relationship of structure to biological activity are now possible. It should be mentioned that none of the degradation products described in this report retain biological activity. It has been reported that certain preparations of intestinal mucosa and skin have hyperglycemic activity. In the event that these materials can be isolated in pure form, structural studies will reveal whether or not the substances are identical to glucagon.

Glucagon is of importance in carbohydrate metabolism, and perhaps with a clearer picture of the nature of glucagon will come an enhanced understanding of the metabolic processes underlying diabetes, and a keener insight into the mechanism of action of insulin.

SUMMARY

The amino acid sequence of glucagon has been determined and the implications of the work were discussed.

SUMMARIO IN INTERLINGUA

Le Sequentia del Amino-Acidos de Glucagon

Le sequentia del amino-acidos de glucagon esseva determinate. Le signification del studio es discutite.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance, during various parts of this work, of E. R. Diller, H. L. Bird, C. T. Pugh, W. A. Tandy, R. G. Scheib, E. E. Logsdon, and C. W. Pettinga.

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A Physiologic Role for Glucagon

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The concept that glucagon is a hormone is rapidly gaining ground in the face of (1) its action on enzyme systems[1, 2, 3] with consistently reproducible effects in these spheres; (2) its biologic production[4] in close relationship to insulin, notwithstanding; (3) its significant deviation from insulin in content[5] and sequence of amino acids and in structural arrangement of its peptides; (4) the strong likelihood of its being controlled and possibly produced in response to one or more trophic hormones;[6, 7, 8] (5) its marked potency, the minimal effective dose,* weight for weight, having been estimated to be roughly one-tenth that of insulin. So potent a naturally occurring agent would be expected to carry more than a fortuitous significance in the body economy.

Common Errors in Interpretation of the Physiologic Role. The normal function of glucagon in the body still remains to be established. Perhaps the greatest handicap to an adequate appraisal of its normal physiology has been the lack of a precise method for its critical assay in blood. As a result, such effects as have been observed have followed what would from the physiologic viewpoint constitute an excessive dosage of the hormone. Such extremes of effect have erroneously been interpreted as representing the true physiologic role of this agent in the body economy. In the light of our findings, even that amount of glucagon contaminating the ordinary injection of commercial insulin (0.3 to 0.5 per cent) would when given at one time seem to us to be a large dose of the hormone. It is doubtful whether under physiologic circumstances glucagon is ever normally poured into the blood stream in such massive dosage as to create such extreme hyperglycemic effects as are produced by the administration of 10 to 20 μg./kg. The extremes of hyperglycemia incidental to this artificial reservoir flooding with the hormone have created the impression that the main function of glucagon in the body is to combat hypoglycemia,[9] particularly that due to an excess of insulin. This view is held by some despite the well-known fact that the adrenal medulla very promptly meets all such exigencies as is evidenced by the early release of lactic acid from the muscles as well as by the sharp train of sympathomimetic symptoms commonly experienced in hypoglycemia arising from insulin. In addition to releasing glucose from the liver, the medullary hormone promptly curtails[10, 11] the utilization of glucose by the tissues, at the same time replenishing liver glycogen by throwing muscle glycogen back into the lactic acid cycle[3, 12] and calling forth protective adrenal cortical function.[13] By contrast, glucagon would seem rather to favor, even if indirectly, the peripheral[14, 15, 16] utilization of glucose; it does not supply lactic acid to the pool and does not discernibly stir the adrenal cortex into activity.[17] Its action is unassociated with the slightest sympathomimetic* symptomatology.[28]

Further difficulty, however, in establishing the true physiologic role of glucagon arises from the fact that the overwhelming hyperglycemia incidental to such unphysiologic dosage from without has ushered in the unwarranted[18, 19] concept that glucagon is under *normal* conditions antagonistic[19, 20, 21] to insulin. In regard to this alleged antagonism, it is to be recalled that glucagon influences enzyme systems relatively unaffected by insulin. The alleged antagonism is under normal conditions largely hypothetical and seems to be based on the large dosages used in the laboratory.

Presented at the Symposium on Insulin, Glucagon and the Oral Hypoglycemic Sulfonylureas sponsored by The Clinical Society of the New York Diabetes Association, Inc., on Oct. 12, 1956.

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* Dr. Mary Root has suggested that by crude estimation in normal rabbits the minimal effective dose of insulin is about one microgram per kilogram; the minimal effective dose of glucagon is from 0.02 to 0.1 microgram per kilogram (personal communication).

* Over 100 individuals were observed by one of the authors (G.E.A.) for evidence of sympathomimetic effects after 10 μg/kg. I.V. None was found in any of these subjects (unpublished data).

A Suggested Interpretation. Our group at the State University of New York has been nurturing the concept that any sustained or exaggerated hyperglycemia created by glucagon is unphysiologic, that during postabsorption glucagon function occurs in brief spurts, probably in response to a demand[16, 22, 4] stimulus, that physiologically, rather than creating the opposite extreme to insulin effect, endogenous glucagon seems to act as a homeostatic stabilizer of blood glucose levels, working concurrently with insulin in a smooth orderly process of homeostasis throughout this period.

The function of endogenous glucagon seems to be most apparent during the state of postabsorption: (1) Throughout the periods between food absorption, glucagon in releasing hepatic glucose into the periphery via the arterial tree would seem to supply the substrate necessary for a continuance of insulin function at the tissue level; (2) the release of hepatic glucose into the arterial tree during these periods would also supply the pancreatic arteries with an increment of glucose for direct stimulation to insulin production[39, 24, 16] at a time when portal blood no longer carries this stimulus in the form of a high titer of glucose absorbed from the intestinal tract.

The ultimate proof that such a relationship between the two hormones actually exists must, of course, await the development of methods for critical assay of both hormones in small quantities of pancreatic venous blood, a method sufficiently facile to permit of precise timing between quantitative changes in the hormones and their effects on target mechanisms. There is even now, however, considerable crude circumstantial evidence to support the validity of this rather revolutionary concept. Much of this evidence springs from careful timing of the known functional effects of both hormones as these are registered in terms of seconds or minutes rather than of hours after their parenteral administration. The purpose of the present communication is to bring into focus this relationship of timing to function.

Mechanisms Involving (a) Arterial Glucose Fluctuations. Contrary to the old concept that glucose is released from the liver in a smooth linear manner, Bondy[23] has shown that during postabsorption, glycogenolysis is expressed in the hepatic veins by levels of glucose which vary grossly and unpredictably from moment to moment. The authors have investigated these aperiodic fluctuations in hepatic venous glucose in dogs by direct needling of an hepatic vein and making blood glucose* determinations at 1-minute and at 15-second intervals.[24] When plotted out these fluctuations are in the form of a bizarre consecutive series of undulations and oscillations, no two of which, in sequence, are alike in amplitude or wave-length. When the circulation time between the hepatic vein and a femoral artery has been established by fluorescine under a Wood lamp, it is possible to identify at the latter point the larger of these fluctuations present in hepatic venous blood (figure 1). In fact, most of the larger glucose undulations and oscillations exhibited in hepatic venous blood are reflected in parallelism (but at lower over-all glucose levels) in the major peripheral arterial tree, despite the central admixture of vena caval with hepatic venous blood (figure 2). In our opinion the peripheral arterial fluctuations so consistently found throughout the entire period of postabsorption, accordingly, represent the peripheral manifestation of spurts of hepatic glycogenolysis; they are the peripheral expression of factors causing glycogenolysis, among which epinephrine and glucagon may be assumed to play the most prominent roles.

The peripheral arterial oscillations and undulations are completely obliterated by hepatectomy, they being replaced by an almost linear descent in blood glucose to the point of exitus of the animal (figure 4). Adrenalectomy itself has no such obliterating effect on the fluctuations either in the hepatic vein or in the peripheral arterial tree, nor does demedullation by heavy dosage of ergotamine[37] (dihydroergotamine tartrate 0.5 mg./kg. i.v.). The existence of these normal postabsorptive fluctuations seems to be completely independent of epinephrine[38] influence. When, however, complete ergotaminization is combined with heavy dosage of one of the arylsulfonylureas, the fluctuations at both sites com-

* The Somogyi-Nelson macromethod for blood glucose was used. The method for true glucose was checked by quantitative fermentation procedure. Readings were made in duplicate. Replicate determinations made on the same blood samples fell within the range of error incidental to the methodology employed, i.e., a standard deviation of from 5 to 5.3 mg. per 100 ml. of whole blood. To favor conservatism in the interpretation of the intra-individual variations found in this study, no variation was deemed to be significant unless it exceeded 8 mg. per 100 ml. of whole blood (an arbitrary increase of 66 per cent above the error actually and repeatedly found in 10 aliquots on individual single specimens of blood). Chi square (goodness of fit) tests carried out on these aliquot series yielded a P. (probability) of 0.99.

All pipettes were carefully checked for correct calibration by the mercury technic. The Evelyn photoelectric colorimeter was used as standard in the colorimetric determinations. After development of the color, the Folin tubes were permitted to stand for 80 minutes, the time required for the establishment of a fixed plateau of color (23). Duplicate readings made 12 hours later showed no significant change.

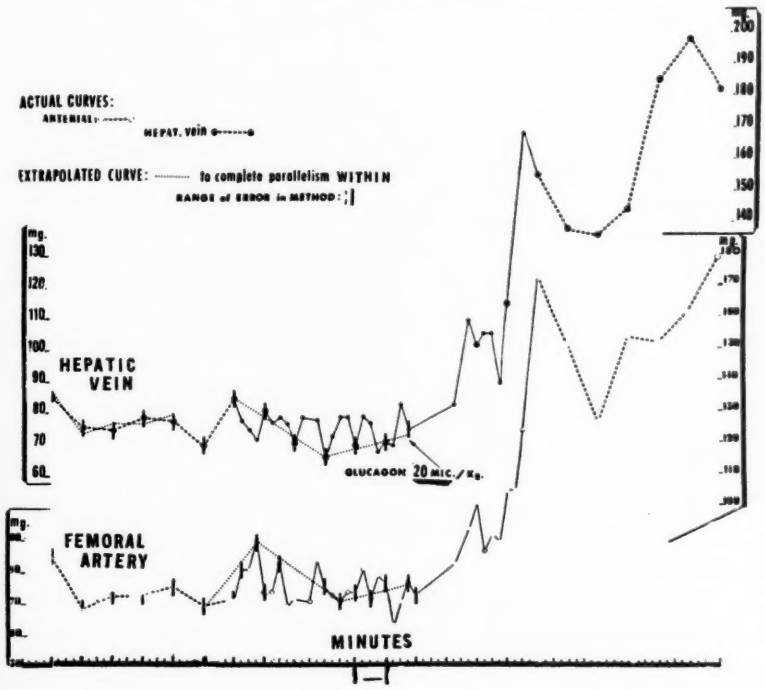

FIG. 1. Glucose undulations and oscillations in hepatic vein of normal dog under sodium-pentothal anesthesia, 14 hr. postabsorptive. Determinations made at 1-minute and some at 15-second intervals. Simultaneous glucose fluctuations in femoral artery with allowance having been made for the circulatory time-lag (10 seconds) between the two points (established by fluorescine under a Wood lamp). Note the parallelism between the larger fluctuations at both sites. The larger waves (undulations) are easily identified in the peripheral artery despite an admixture of hepatic venous with caval blood entering the arterial tree. The identity of the smaller oscillations is largely wiped out by passage of the blood through the cardio-pulmonary channels. In areas where parallelism is not apparent, it is to be noted by extrapolation that when half of the possible mean error in the glucose determinations (the standard error being 5.3 mg. per 100 ml.) is plotted, the major fluctuations at both sites are parallel within this range of error. Adapted by courtesy of Proceedings of Society for Experimental Biology and Medicine, 92:341, 1956.

pletely disappear[25] and are replaced by a linear descent in hepatic venous and in arterial blood glucose to the point of exitus, just as in the completely hepatectomized animal (figures 3 and 4). There is, however, one outstanding difference between the states of the two dogs: in the ergotaminized-Orinase-treated animal, the administration of a relatively small dose of glucagon[25, 26] by vein (3 to 5 μg./kg.) at any point of the glucose descent immediately causes a temporary resumption of the normally occurring pretreatment undulations and oscillations (figure 3) both centrally and peripherally, with an over-all rise in blood glucose. Presumably, the animal had been deprived of both important mechanisms normally capable of producing glycogenolysis, (1) restriction of epinephrine effect by heavy ergotaminization and (2) interference at some step involved in the production of glycogenolysis via glucagon. Houssay[27] has noted the extreme sensitivity of the adrenalectomized animal to the arylsulfonylureas, the combination leading to death in hypoglycemia unless glucose is administered. The authors have narrowed down the influence of this adrenal insufficiency on sensitivity to a deprivation of the adrenal medulla and chromaffin tissue itself, for heavy ergotaminization produces the very same phenomenon of sensitivity with the adrenal cortex present[28] and intact. Since under these circumstances the postabsorptive type of undulation and oscillation is immediately restored both in hepatic venous[26] and in peripheral arterial blood by a small dose of glucagon, they believe that this hormone may be responsible in the first place for the existence of the normal postabsorptive fluctuations found in dog and man.

(b) *Venous Fluctuations.* In another communication[24] it was pointed out that venous glucose fluctuations similar to those in the arterial tree also occur normally throughout postabsorption both in man and in the intact dog (figure 2). It is suggested that the brief sharp descents in these venous undulations and oscillations indicate that free glucose has left or is in the process of leaving the small arterial radicles (diffusion). The egress of such glucose from the vessels is favored by the transient existence of an increased glucose gradient between the vascular tree and the tissues as a "zero point." Endogenous insulin acting at the tissue surfaces to accelerate the transfer of the glucose across the cellular membranes further accentuates the effect of the entire gradient in the direction of the cells. Within 10 to 20 seconds, however, after the fall in venous glucose reaches its nadir, there occurs a sharp rebound in venous glucose, which approaches but usually does not entirely

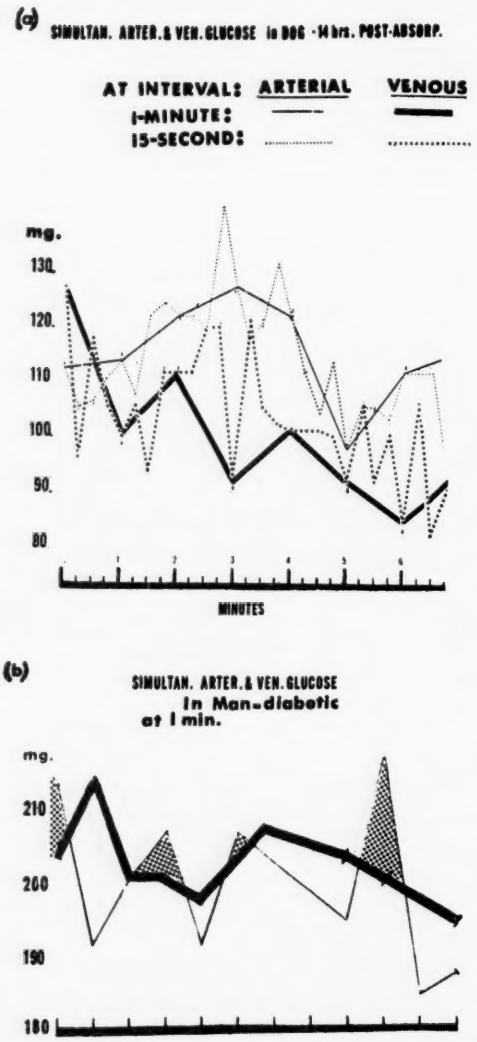

FIG. 2. (a) Arterial (femoral) and simultaneous venous (femoral) blood glucose determinations at 15-second intervals in normal dog, 14 hours postabsorptive (anesthetized with pentobarbital-sodium). Allowance made for venous lag, established by fluorescine under Wood lamp. Note aperiodicity and sharpness of oscillations, each requiring about 30 seconds for completion of both phases. When glucose determinations are made at 5-second intervals and these readings are plotted, oscillations typical of the 30-second fluctuations are produced. Note that the venous fluctuations are consistently opposite to the arterial. When the readings are plotted at 1-minute intervals, undulations with wavelengths of 2 to 5 minutes are produced similar to those in cut (b), readings from brachial artery and venous returns at 1-minute intervals in man, 14 hours post-absorptive (not under anesthesia). Adapted by courtesy of Science 122:458, 1955 and Pennsylvania Med. J., March 1956.

achieve[24] the original level of venous glucose whence the fall was initiated (see heavy black lines in figure 2a). This ascending limb of venous glucose is less readily accounted for than are the falls.* Whether or not it can be proved that free glucose leaves and returns to the vascular bed usually in less than one minute's time, the authors have beyond reasonable doubt established the fact that venous glucose level changes sharply and significantly from moment to moment in an oscillatory manner, each complete oscillation (down and up) requiring approximately 30 seconds[24] of time.

The Promptness and Sharpness of Both Hormonal Effects. It is well recognized that the glycogenolytic effect of glucagon administered into the blood stream is

* Presumably, all of the free glucose passing from the capillaries into the tissue spaces and thereby producing the observed venous falls does not continue on to cross the cellular membranes, for *parallel* changes (falls) in serum potassium and inorganic phosphorus do *not* accompany each of these sharp declines in venous glucose. Most of the free glucose leaving the vascular tree seems almost immediately to return from the tissue spaces back into the venous radicles as the new temporary "zero points." This sharp reversal of flow would require a sharp change in the direction of glucose gradient toward the relatively lowered glucose of the venous radicles.

The height reached by each ascending limb in the successive oscillations usually falls short of the level of origin of each respective preceding falling limb (see venous curves in figure 2a). When these successive crest points are plotted out, the descending curve of these discrepancies runs parallel with a curve of serum potassium and inorganic phosphorus representing an even and slow fall in these two factors in the serum. In a normal dog, heavily ergotaminized, a rear extremity was completely amputated, save that the femoral arterial blood supply was left intact with the body, the venous return being deviated into a collecting vessel, and accordingly, not allowed to return to the body. When in this extremity all sulfhydryl-obligate enzyme systems (including the hexokinase)[28] are poisoned by a weak solution of $HgCl_2$ perfused via the artery, the deficits in the ascending limbs of the venous oscillations promptly disappear, glucose again attaining the starting levels. Venous blood glucose (not arterial, of course) in addition shows an over-all sharp rise. The resupplying of fresh -SH groups to the extremity via the constant flow of fresh arterial blood is attended by a resumption for a short period of a return of the deficits originally exhibited in the ascending limbs of the venous oscillations. Glucose determinations were made at 5-second intervals.

It is, accordingly, thought by the authors that the venous discrepancies usually found represent such glucose as having left the vascular tree goes on from the intercellular spaces, crosses the cellular membranes and is there retained in phosphorylation. The rest of the free glucose in the spaces promptly returns to the venous tree. The parallelism existing between the curve of these glucose discrepancies and that of falling potassium and inorganic phosphorus lends support to this concept to explain the deficits observed.

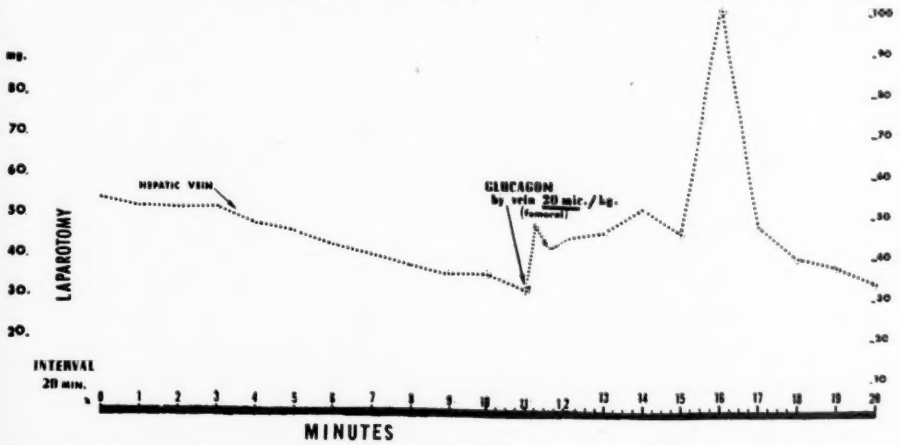

FIG. 3. In a normal dog treated for 42 days with heavy dosage of arylsulfonylurea tolbutamide, 200 mg./kg./day, p.o.), laparotomy was performed under sodium-pentobarbital, 14 hours postabsorptive. Direct sampling of hepatic venous blood at 1-minute intervals revealed an absence of all the glucose fluctuations normally present in this blood. When the animal was also heavily ergotaminized (di-hydroergotamine 0.5 mg./kg. i.v.), there occurred in addition a rapid decline in hepatic venous glucose values, practically a linear descent to the point of critical blood-glucose deficiency, precisely as in the completely hepatectomized animal. Glucagon administered i.v. at this point produced an immediate return of the normal pre-treatment hepatic venous fluctuations in exaggerated form with an overall rise in blood glucose. (Even a dose of glucagon as small as 3 μg./kg.) has repeatedly been demonstrated to produce this effect.) Presumably, the animal had been deprived of both available agents for hepatic glycogenolysis, epinephrine and glucagon. The implication is that it is glucagon which causes the normal postabsorptive glucose fluctuations in hepatic venous blood.

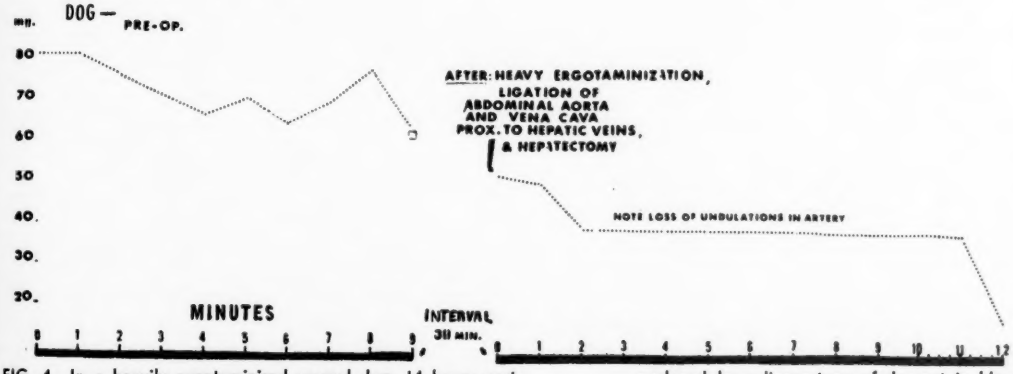

FIG. 4. In a heavily ergotaminized normal dog, 14 hours postabsorptive, under pentobarbital-sodium anesthesia, ligation (proximal to the liver) of the abdominal aorta and inferior vena cava, followed by complete hepatectomy (the equivalent of an abdominal evisceration) immediately causes a complete loss of glucose fluctuations in the arterial supply to the forepaw. These had been present immediately before the ligations. They are replaced by a linear type of descent in blood glucose to the point of exitus of the animal. It had been previously repeatedly demonstrated that ergotaminization alone has no effect whatsoever on the presence of the normal postabsorptive fluctuations. Glucose determinations at 1-minute intervals.

Adapted by courtesy of American Journal of Clinical Nutrition 4: 1956.

prompt. But even in massive dosage, it is evanescent unless the hormone is continuously injected. The sharp brief episodes of glycogenolysis normally registered in hepatic venous blood throughout the entire period of postabsorption are typical of the series of consecutive sharp evanescent rises in arterial glucose restored by a small dose of glucagon in a dog that had been deprived by drugs of his ability to release glucose from the liver (q.v. above). The question arises as to whether the normally occurring consecutive spurts of glycogenolysis and their distal reflections in the form of arterial undulations and oscillations are accompanied by evidence of corresponding phases of insulin effect at the tissue level, viz., a fall in venous glucose and an in-

crease in a-v difference at these distal points. To entertain with reasonable justification any such concept one would be obliged to demonstrate that during postabsorption the action of insulin at the tissue level also occurs in brief spurt-like episodes, a series of which ultimately produces by cumulative action a gradual fall in blood glucose. It would also have to be disproved that during this period the action of insulin is a sluggish and desultory phenomenon.

Stadie[29, 30, 31] and his group produced the first experimental data to suggest that the old notion that the action of insulin at the tissue level is necessarily a sluggish time-belabored phenomenon. There seems to be so powerful a physico-chemical affinity between muscle-tissue and insulin, that the fixation of insulin by the tissue surfaces is immediate and irreversible within a second or two after contact.

The authors have gathered considerable in vivo experimental data to suggest that very promptly after injected insulin reaches its target tissue the level of venous glucose returning from this region falls. There evidently is in normal man and dog little or no latent period between the fixation of insulin by the tissue and the appearance of evidence of glucose transfer. (The term "transfer" is used in an all-inclusive sense and embraces the diffusion of free glucose from the vessels into the intercellular spaces as well as its transport across cellular membranes.) In man and in the dog, within one minute, and often within 20 seconds, after the administration of glucagon-free insulin* by vein, there occurs a sharp[24] fall in the glucose level of blood withdrawn from the same vein. Assuming that the injected insulin would have had to be carried through the cardio-pulmonary channels and thence into the arterial tree to reach its muscle target, one would be obliged to conclude that in vivo with a 20-second timing, there is little or no latent period possible. The effect of insulin on initiating glucose transfer is practicially immediate. This point is further borne out by the fact that administration of the insulin by artery produces an even more prompt fall in returned venous glucose (6 seconds or less).

Levine[35,36] et al. have cleared another real obstacle to plausibility of a concept that insulin effects the transfer of glucose across cellular barriers with great rapidity by demonstrating that the transfer does not, as formerly believed, depend on preliminary phosphorylation of the transported glucose. It has been shown beyond reasonable doubt that the steepness of the glucose gradient toward the "zero point" in the tissues is a most important determinant[31, 34] of the rate of transport across membranes. Park et al.[34] have, moreover, effectively shown by in vitro experiments that free glucose crossing the cellular membranes is so rapidly phosphorylated that under the influence of insulin intracellular free glucose cannot be detected until the concentration of glucose in the medium is raised to 2,000 mg./100 ml. Then the effect of insulin increases the transport of glucose beyond the capacity of the hexokinase system within the cells to react with it. Insulin acts to sharpen strikingly the effectiveness of any glucose gradient. The steady aperiodic releases of glucose into the arterial tree during postabsorption would seem to supply the gradient.

The Inadequacy of Venous Fluctuations as a Measure of Hepatic Glycogenolysis. No two venous glucose determinations on blood samples from different areas of the body, even from homologous sites, will be found to be simultaneously identical,[24] save by accident. This phenomenon of variation from point to point probably reflects the fact that the utilization of glucose varies from tissue to tissue depending on many circumstances, including the metabolic activity and the bulk of the respective tissue. These are reflected in change in venous values from point to point. By contrast, fluctuations in arterial glucose represent increments of glucose arising directly from the liver and only mirrored in the periphery.[24] They are more uniform from site to site. They are, accordingly, a more direct and sensitive index of the aperiodic pulsatile episodes of glycogenolysis constantly occurring throughout postabsorption.

A Role for Endogenous Glucagon. In the periods between food absorption, the repetitive and consistent sequence of a jet-like pouring out of glucose from the liver into the arterial stream (probably a glucagon

* One of the authors (G.E.A.) has had an opportunity to observe this phenomenon of rapid fall in venous glucose after glucagon-free insulin (i.v.) in many individuals, both normal and diabetic, who were exposed to the so-called "Six-minute Test"[32, 33] for sensitivity to insulin. In brief, the test measures the promptness and sharpness of the body's response to insulin administered by vein. An insulin which has been freed of its glucagon is used in order to prevent early masking of an otherwise sharp insulin effect. A single dose of 3 units of this insulin is given by vein in the fasting state. Venous glucose levels are determined in the fasting state and at 1 or 2, 4 and 6 minutes after the administration of the insulin. Unless the fasting level happens to be very low to begin with, the normal individual exhibits with the 6-minute period a fall in blood glucose of 19 per cent with a differential of ± 7 per cent. These criteria have been established in a study of over 600 of these tests to date and have established beyond reasonable doubt the fact that insulin's action in peripheral tissue during postabsorption is very rapid.

effect), immediately associated with an episode of fall in venous glucose (essentially an insulin effect) and a widened a-v difference, probably incidental to the combined effects of both hormones, suggests that during postabsorption there is an orderly relationship between factors causing the observed arterial rises and those causing the venous falls in glucose. The consistent precise simultaneity of these two opposite trends in blood glucose is against their being a phenomenon of chance (figure 2).

Ingested glucose in the insulin-normal animal supplies a satisfactory peripheral glucose gradient to promote the transfer of free glucose across cellular membranes. It is well accepted that the same arterial flooding on reaching the pancreas triggers the production of insulin by[39, 24, 16] that organ. It is the opinion of the authors that during periods *between the absorption of food* when insulin is obviously still actively functioning in the body, endogenous glucagon serves to establish a similar, if less marked, peripheral glucose gradient, which also in these times supplies an arterial glucose stimulus to the pancreas for the production of insulin.

DISCUSSION

A plotting of the arterial spurts present[24] during postabsorption shows that these are in orderly manner followed by brief episodes of tissue utilization or, at least, by episodes of egress of glucose from the vessels. Each rise in arterial glucose is promptly associated with a sharp fall in venous glucose and a brief period of positive a-v difference (figure 2). Serum potassium and inorganic phosphorus assume a trend which is slowly downward in a linear manner without the "ups and downs" as seen in their accompanying glucose curves. With the passage of time postabsorptively, the mean of all arterial fluctuations approaches and ultimately becomes practically identical with the mean of all venous fluctuations, i. e., there actually seems to occur a progressive depletion in the amount of glucose in the central stores available to the periphery for tissue utilization. It is, however, noteworthy that at no time is there an absence of the episodes of a-v difference, small as these may ultimately become. Endogenous glucagon, accordingly, serves as a homeostatic agent to smooth out the approach to the depleted state without necessitating the calling forth of adrenal medullary action with its explosive sympathomimetic consequences.

SUMMARY AND CONCLUSIONS

By its orderly promotion of glucose distribution throughout the body and by its indirect triggering of endogenous insulin function, glucagon assumes an important role in the economy of glucose. As one of its functions, glucagon appears to serve during postabsorption to correlate the production of glucose and insulin at source with the peripheral utilization of glucose by the tissues, supplying at the tissue level a glucose gradient which would otherwise be lacking during this period of nonabsorption. Intrinsic glucagon, accordingly, during postabsorption not only favors the production of insulin at source by supplying the stimulus to such production, but it potentiates the peripheral utilization of glucose by establishing in a peripheral glucose gradient the substrate required for local transport. Its action normally meshes in with and is, accordingly, synergistic[41, 40, 22, 16] with that of insulin. Endogenous glucagon thus seems to be a homeostatic stabilizer of blood glucose during periods between the absorption of food.

The biologic production of glucagon for what apparently is a consistent and orderly effect on enzyme systems warrants glucagon's being considered a hormone.

Under normal conditions, endogenous glucagon is an adjuvant and an orderly synergist to insulin function. This concept of a purposeful normal function for glucagon deserves further exploration.

SUMMARIO IN INTERLINGUA

Un Rolo Physiologic de Glucagon

Glucagon promove un ben-ordinate distribution de glucosa in omne partes del corpore e initia indirectemente le function de insulina endogene e assi executa un rolo importante in le economia de glucosa. Il para que le functiones de glucagon include que illo servi durante le postabsorption a correlationar le production de glucosa e insulina al puncto de lor origine con le utilisation de glucosa per le tessutos peripheric, providente in le tessutos un gradiente de glucosa que non existerea alteremente durante iste periodo de nonabsorption. Durante le periodo de postabsorption, glucagon assi representa non solmente un factor que promove le production de insulina al puncto de su origine per provider le stimulo de iste production, sed illo etiam effectua un potentiation del utilisation peripheric de glucosa per establi in le gradiente peripheric de glucosa le substrato requirite pro le transporto local. Su action se ingrana normalmente con le action de insulina (e es, per consequente, synergic con illo). Assi glucagon endogene pare esser un stabilisator homeostatic del glucosa del sanguine durante le periodos inter le absorption de nutrimento.

Le production biologic de glucagon pro apparentemente uniforme e ben regulate effecto in systemas enzymatic justifica nos a considerar glucagon un hormon.

Sub conditiones normal, glucagon endogene es un adjuvante e un ben-ordinate synergico in le function de insulina. Iste concepto de un normal function physiologic de glucagon merita explorationes additional.

ACKNOWLEDGMENTS

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The authors desire to express their gratitude to Mrs. Agnes C. Dann, R.N., for her invaluable assistance in carrying through all of the blood glucose determinations involved in this study.

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Relations of Taste and Odor to Appetite

Traditionally physiologists and psychologists both have studied the sense organs and their relation to the total central nervous system. Progress in the visual and auditory spheres has been tremendous compared to that in the chemical senses.[1]

Adrian's electrophysiologic research in the chemical senses has enabled him to find characteristic shapes on a photographic reproduction of the discharge obtained from leads off the mitral cell layer of the olfactory bulbs. Substances such as xylol, pyridine and eucalyptus, when present in the air, can be identified by the curves on the record.[2]

"Although Adrian hesitates to conclude that the brain identifies the smell by the same criteria, he states that it would be easy on this basis to see how a great variety of smells might be distinguished without the need for very great variations in the receptors. If this is so, Adrian feels that it would be possible to relate olfactory discrimination both to auditory and to visual discrimination. Tones are distinguished by the general regions of the basilar membrane which is excited; visual scenes, by the detailed patterns. Smells seem to be distinguished by a combination of detailed pattern and general region. But when all this is clear, the central problem of the nervous system will still be to explain how the spatio-temporal patterns of incoming discharges are dealt with by the brain."[3]

At this time we cannot expect the sensory physiologist to help us with problems at the psychological level of integration. The sensory psychologist is only gradually learning to measure the four tastes: bitter, sweet, sour, and salt. Perception of a taste involves, in addition, odor, touch, and temperature. Appetite probably also involves the state of hunger (including the emotional state), the appearance of the food, and previous habits of eating. The voluminous literature on these problems gives us very little help in understanding why some people have have either hypo- or hyperphagia. The factors governing food intake appear to be related principally to the total organization of the individual rather than to the anatomy or the functional efficiency of the 9,000 taste buds on his tongue or the nerve pathways conducting the impulses to the brain.

From the book *Modern Nutrition in Health and Disease* edited by Michael G. Wohl, M.D., and Robert S. Goodhart, M.D. Philadelphia, Lea & Febiger, 1955, Chapter "The Psychology of Appetite" by Henry W. Brosin, M.D., pp. 77-78.

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The Pathologic Effects of Large Amounts of Glucagon

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The work of Ingle[1] and Cavallero[2] has shown that glucagon will produce a temporary or mild glucosuria in partially depancreatized animals and in animals pretreated with cortisone, but a significant diabetogenic action of glucagon administered alone to intact rats has not previously been shown.

The results of early attempts made in our laboratory to show a diabetogenic action of the pancreatic hyperglycemic factor were disappointing. We reinvestigated the problem using much larger doses of glucagon attempting, at the same time, to prolong its activity by suspending it in corn oil and administering it subcutaneously at eight-hour intervals. Glucagon administered under these conditions appeared to exert a profound effect.

Figure 1 shows the average changes in weight and food intake of intact male controls injected with corn oil, and of normal male rats injected three times daily with 300 *u*g. of glucagon (Lilly, lot no. 258-234B-33) suspended in corn oil. Weight changes are shown for a second set of controls limited to the amount of food consumed by the glucagon-treated animals.

It is apparent that the glucagon-treated rats consumed much less food than the controls and lost weight rapidly. The weight loss cannot be completely attributed to the reduction in food intake, as the pair-fed controls lost much less weight.

The glucagon-treated animals were not glucosuric but animals similarly treated and encouraged to eat bread, frequently showed a transient but intense glucosuria. This stimulated us to investigate the effect of glucagon in force-fed rats.

Male Wistar rats weighing 150 to 160 gm. were fed by stomach tube at 8:00 a.m., 4:00 p.m., and 12:00 m., the high carbohydrate diet described by Reinecke, Ball and Samuels.[3] The volume administered was slowly increased until the tenth day; thereafter each rat received 10 ml. of the fluid diet, containing 6 gm. of solids at each feeding. Five animals served as controls and were injected with corn oil. The remaining five rats received subcutaneously at six-hour intervals a total of 1.2 mg. of glucagon daily. The glucagon was suspended in corn oil* in a concentration of 3 mg. per ml.

Figure 2 shows the average daily (a) glucose excretion, (b) urinary nitrogen excretion, and (c) body weight changes in the force-fed controls and in the glucagon-treated animals.

It is evident that the control rats excreted no glucose and gained weight during the experimental period. The glucagon-treated animals excreted approximately 4 gm. of glucose daily. Their urinary nitrogen excretion was nearly twice that of the controls and they lost weight rapidly. The blood sugar levels of the glucagon-treated animals remained between 350 to 450 mg. per cent throughout the day, while the controls showed only a slight increase in blood sugar concentration after each feeding.

During the experimental period the control rats remained in excellent health while the treated animals became emaciated and ill, with only two of the five rats surviving a seven-day period.

A second experiment was then performed in an attempt to produce permanent diabetes with glucagon. In this experiment twenty force-fed rats were used—ten control rats and ten glucagon-treated. The results substantiated those obtained in the first experiment. Intense glucosuria and hyperglycemia developed rapidly with some animals excreting as much as 10 gm. of glucose daily. However, this investigation was difficult to carry out. The force-fed control rats thrived but the glucagon-treated animals became increasingly ill until after a week they required constant attention. The treated rats suffered gastrointestinal disturbances and their stomachs frequent-

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Banting and Best Department of Medical Research, University of Toronto, Canada.

* We have recently found that it is unnecessary to suspend the glucagon in oil. A neutral saline suspension is equally effective when administered subcutaneously.

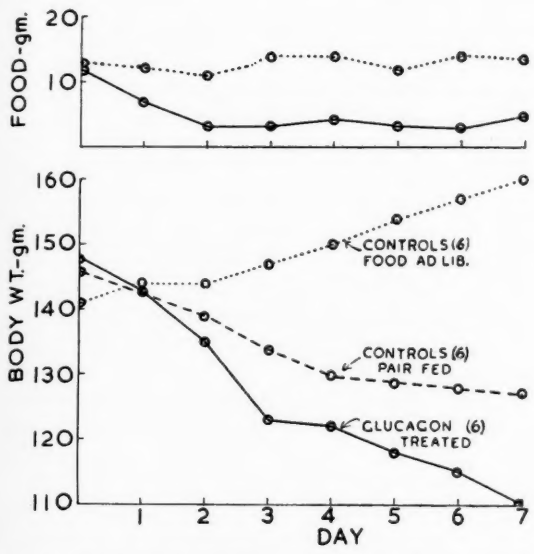

FIG. 1. Changes in the weight and food intake of male control rats injected with corn oil and of rats injected subcutaneously every eight hours with 300 gamma glucagon in oil.

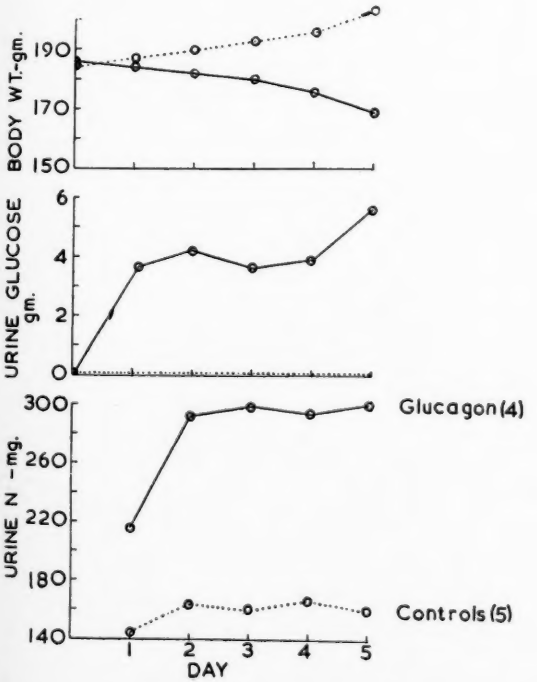

FIG. 2. Urinary nitrogen and glucose and body weight of force-fed controls and of force-fed rats injected every six hours with 300 gamma of glucagon.

ly became so distended that it was necessary to aspirate the contents. In other instances the animals would suddenly lapse into a state resembling diabetic coma with blood sugar levels between 800 to 900 mg. per cent; this latter condition responded dramatically to insulin therapy. We succeeded in maintaining only two force-fed rats on glucagon for a period of ten days. The animals remained glucosuric and hyperglycemic for six days following the cessation of all treatment. We feel that if treatment can be maintained for a longer time permanent diabetes may be produced.

In two normal dogs studied with Dr. James Campbell, glucagon in oil produced transient hyperglycemia and mild glucosuria. Since the supply of glucagon was limited the treatment of these animals was neither intensive nor prolonged. The dogs were sacrificed after one week.

Histological examination of pancreas revealed degranulation of the β-cells. The extractable insulin content of the pancreas was found to be only 15 per cent of the normal.

The histological appearance of the pancreatic β-cells in the glucagon-treated rats has been variable. In some instances these cells show degranulation and hydropic degeneration, in other cases the β-cells appear to be more intensely granulated than normal. In both dogs and rats, glucagon consistently produces marked degranulation and atrophy of the acinar cells. The significance of this observation is not evident.

Although the glucagon used in these investigations was highly purified, it contained considerable amounts of other proteins. The possibility that the diabetogenic effect was due to an unknown contaminant had to be considered. However, we have since found that pure crystalline zinc glucagon has the same diabetogenic action; thus we attribute our initial results to the action of glucagon alone. The results of these investigations clearly show that under our experimental conditions glucagon possesses marked diabetogenic properties when administered to force-fed rats and to dogs fed ad libitum. It is not known, at this time, that a permanent diabetes can be produced with the pancreatic hyperglycemic factor.

The mechanism(s) through which glucagon acts to produce diabetes is unknown. The effect of this substance on extrahepatic carbohydrate utilization remains a controversial subject. Studies carried out in vitro by Candela[4] and Snedecor, De Meio and Pincus[5] indicate that glucagon inhibits the stimulating effect of insulin on glucose uptake and glycogen synthesis by the isolated rat diaphragm. However, Smith working with Dr. F. G.

Young[6] has been unable to confirm this observation and Clarke of our department finds that the stimulating effect of insulin on glucose uptake by the rat diaphragm in vitro is enhanced if the animals are pretreated with glucagon.

Drury, Wick and Sherrill[7] have reported that the hyperglycemic factor slightly inhibits the disposal of blood glucose in eviscerated rabbits while Ingle, Nezamis and Humphrey[8] claim that it has no effect on glucose utilization in eviscerated rats. Studies of a-v differences lead Elrick et al[9, 10, 11, 12] to conclude that glucagon enhances peripheral utilization in normal human beings and in normal or depancreatized dogs. The observations made by Elrick in normal humans have been confirmed and extended by Van Itallie, Morgan and Dotti.[31] Bondy and Cardillo[14] could find no evidence of inhibition of glucose utilization in glucagon-treated humans. In our own department Dr. Margaret Henderson working with Dr. G. Wrenshall, has found in one experiment using C^{14}-labeled glucose an increase in the utilization of glucose following the administration of glucagon to depancreatized dogs.

Although a definite conclusion cannot be reached, the experimental data presently available do not favor the view that the genesis of glucagon diabetes in the intact animal is due to a reduction in peripheral glucose utilization.

Our own data indicate that increased gluconeogenesis contributes to the glucagon-induced diabetes. Figure 3 shows the average urinary nitrogen excretion of intact fasting control rats and of comparable animals treated with glucagon. It is apparent that the fasting glucagon-treated animal excretes about 40 per cent more nitrogen than the controls. Under these conditions no glucosuria or hyperglycemia occurs. The glucagon-induced increase in nitrogen excretion can be almost completely attributed to the increase in urea excretion.

Figure 4 shows the changes in the amino acid and sugar levels in the blood of controls and in rats injected with 1 mg. of glucagon suspended in saline. The blood amino acids fell much more rapidly in the glucagon-treated animals and remained lower throughout the five-hour observation period. The blood sugar rose immediately after glucagon administration but fell within three hours to normal levels. It does not appear that the glucagon-induced fall in blood amino acids can be attributed to an increase in the rate of peripheral utilizations since urinary nitrogen excretion also increased during this period.

In some respects the changes induced by glucagon administration are similar to those induced by adrenal glucocorticoids. The possibility that the marked increase in gluconeogenesis following glucagon administration was due to stimulation of the adrenal cortex was investigated.

Figure 5 shows the total nitrogen and urea nitrogen excreted by adrenalectomized glucagon-treated rats and adrenalectomized controls. The food intake of the controls was limited to the amount consumed by the treated animals. It is apparent that the glucagon-treated ad-

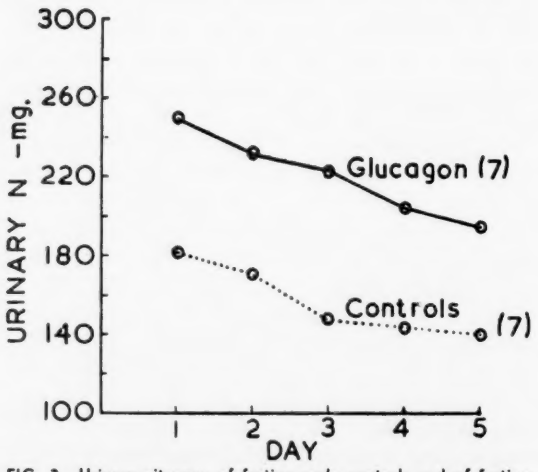

FIG. 3. Urinary nitrogen of fasting male controls and of fasting rats injected every eight hours with 400 gamma of glucagon.

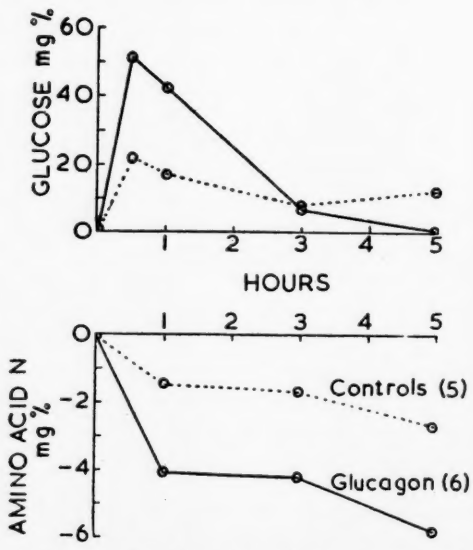

FIG. 4. Changes in the blood amino acid and sugar levels of male control rats and of comparable animals given one subcutaneous injection of 1 mg. glucagon suspended in neutral saline.

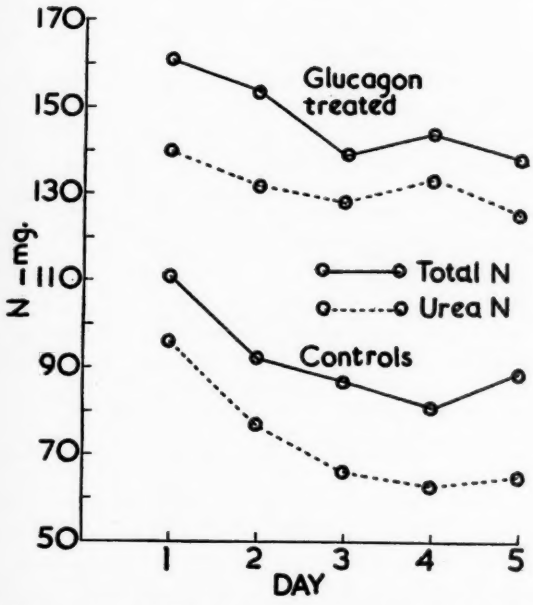

FIG. 5. Urinary nitrogen and urea nitrogen excretion of seven male adrenalectomized rats (maintained on saline) injected with 400 gamma of glucagon every eight hours and of seven adrenalectomized pair-fed controls.

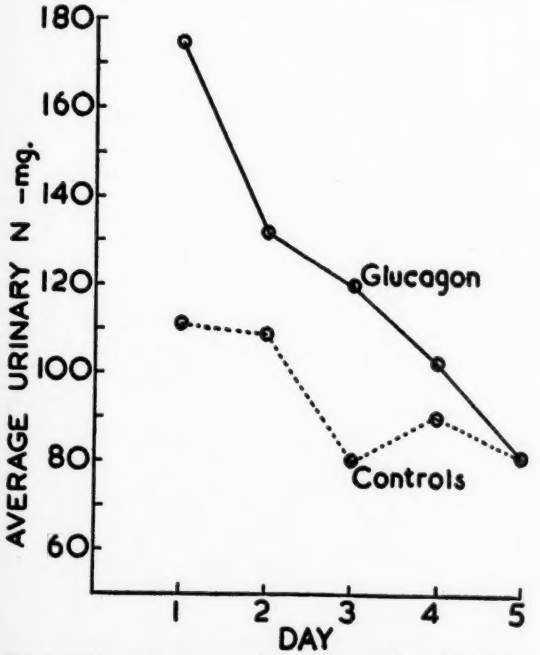

FIG. 6. Urinary nitrogen excretion of five fasting adrenalectomized rats injected with 400 gamma glucagon every eight hours and of five fasting adrenalectomized controls.

renalectomized rats excreted about 50 per cent more nitrogen and urea than the controls.

Figure 6 shows the average urinary nitrogen excretion of fasting adrenalectomized rats treated with glucagon and of the fasting controls. The glucagon induced a marked increase in nitrogen excretion the first day but the effect became progressively less until on the fourth day there was no significant difference between the amounts of nitrogen excreted by each group. It will be recalled that in the fasting intact rat the glucagon-induced increase in nitrogen excretion was undiminished at the end of five days (see figure 3).

The results of these last two experiments indicate that the catabolic action of glucagon is not mediated by way of the adrenal gland. However, the effect of glucagon on the catabolism of amino acids during the fasting state is partly dependent upon the presence of adrenal cortical secretions. Recent experiments have shown that the diabetogenic actions of glucagon and cortisone are markedly synergistic. Rats treated with both substances develop severe diabetic symptoms despite a 75 per cent reduction in their food intake. Weight loss is precipitous and death occurs in from five to seven days.

SUMMARY

The data presented show that glucagon when administered in large amounts has a marked diabetogenic action in force-fed rats. It produces intense glucosuria, hyperglycemia and weight loss. Preliminary experiments indicate that glucagon has a diabetogenic action in dogs fed ad libitum. The specific mechanisms responsible for glucagon diabetes are not known, but the data strongly indicate that glucagon induces a marked increase in gluconeogenesis and suggest that overproduction of glucose contributes to this phenomenon. The protein catabolic effect of glucagon is not mediated by the adrenal glands although a synergy between the actions of adrenal cortical secretions and glucagon appears to exist.

SUMMARIO IN INTERLINGUA

Effectos Pathologic de Grande Quantitates de Glucagon

Le datos presentate monstra que glucagon administrate in grande quantitates ha un effecto marcatemente diabetogene in rattos alimentate per compulsion. Illo produce intense grados de glucosuria, hyperglycemia, e perdita de peso. Experimentos preliminar indica que glucagon ha un effecto diabetogene in canes alimentate ad libitum. Le mechanismos specific que es responsabile pro diabete a glucagon non es cognoscite, sed le datos indica fortemente que glucagon induce un marcate augmento de

gluconeogenese, e illos pare suggerer que un hyperproduction de glucosa contribue a iste phenomeno. Le effecto de glucagon in le catabolismo proteinic non es mediate per le glandulas adrenal, ben que il pare que il existe un forma de synergismo inter le actiones del secretiones adrenocortical e de glucagon.

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GROUP DISCUSSION

CHAIRMAN BRANDALEONE: Thank you, Dr. Salter. The three previous papers on glucagon will be discussed by Dr. Joseph L. Izzo of Rochester, New York.

JOSEPH L. IZZO, M.D.: Three years ago crystalline glucagon was first prepared; now, its chemical structure has been determined. This is a singular accomplishment by Dr. Bromer and his colleagues at the Lilly Research Laboratories. As Dr. Bromer points out, it should now be possible to study the relation of the structure of glucagon to biological activity. I am not qualified to discuss critically the actual data presented. However, the methods which have been employed are standard, and the results seem straightforward. One thing which has impressed me is the plethora of OH groups in the single straight chain glucagon molecule. Does this have any biological significance? Perhaps Dr. Bromer can tell us if they are essential for activity of the hormone, as they are in insulin.

The brilliant advances in the chemistry of glucagon serve to point up the poignant and still unsettled question of the physiologic or pathologic importance of glucagon. Since a convincing example of a deficiency picture has not yet been established, either clinically or experimentally, its hormonal status has not been proved to the satisfaction of all. Dr. Anderson considers glucagon a hormone whose physiologic role is that of an adjuvant and orderly synergist to insulin function. This is said to be accomplished by glucagon triggering hepatic glucose output through its stimulation of phosphorylase activity and thereby providing the stimulus for insulin production in the postabsorptive state. This represents an extension of an hypothesis which, I believe, was first proposed by Bürger, one of the most distinguished students of this substance, several years ago. Unfortunately, as Dr. Anderson himself admits, the hypothesis can be neither proved nor disproved by studies the type of which were just presented. Whether or not the irregular undulations in hepatic glucose output demonstrated by Dr. Anderson as well as others are attributable to glucagon is a moot point. It would be of interest in this respect to know what happens to these "hepatic glucose spurts" in the animal deprived of intrinsic glucagon either by pancreatectomy or by selective destruction of the α-cells of the pancreas,

supposedly the principal source of glucagon. Furthermore, what relation does the pituitary-adrenal axis have to these "spurts"? Are they present in the hypophysectomized or adrenalectomized animal?

It is generally agreed that glucagon is glycogenolytic. However, it is debatable whether or not glucagon has any peripheral action. Some investigators have reported a mild inhibition of peripheral glucose utilization by glucagon, others have noted no effect, while still others have observed an enhancement of glucose utilization. The divergence of results may, to a certain extent, depend upon the different experimental conditions of the investigators. At any rate, it does not appear at the present time that the peripheral action of glucagon, if any, is a major one. The lack of a strong peripheral effect has led some to question seriously the diabetogenic potential of glucagon. Nevertheless, Dr. Salter and his associates have demonstrated convincingly that massive doses of glucagon are indeed diabetogenic for rats and dogs. It is possible that if they had been able to continue their experiment long enough permanent diabetes might have been produced in these treated animals. Of course, they used tremendous quantities of glucagon, but it is conceivable that smaller doses over a longer period of time, particularly now that the pure crystalline hormone is available, may achieve the same result.

The work of the Toronto group is extremely interesting to us because we have been working independently along somewhat similar lines in the human. We have been investigating the effects of repeated daily intramuscular administration of glucagon (at eight hour intervals around the clock) on organic and inorganic metabolism in stable and unstable diabetic patients under optimal and suboptimal regulation with diet and insulin. I should like to point out that on a per unit weight basis the doses we have been using are much smaller than those used by Dr. Salter. Diabetics were selected for the initial studies for two reasons: one, diminished to absent islet cell reserve would reduce to a minimum the possibility of obscuring the metabolic response to glucagon by secondary stimulation of endogenous insulin production; and, two, the role or importance of glucagon in diabetes has not been clearly defined. It is a pleasure to report that our data are in good agreement with those of Dr. Salter. We have also observed the protein catabolic action of glucagon. Time does not permit a detailed report of our studies, but an example of the type of study in progress and data obtained is illustrated by the following case.

The patient was a nineteen-year-old white girl with unstable diabetes of recent onset. Two months prior to admission to the Metabolism Ward she was admitted to the hospital in diabetic acidosis. History revealed that for two years she had had symptoms suggestive of diabetes mellitus but had not consulted a physician. During hospitalization the acidosis was controlled and diabetes regulated by diet and insulin. Following discharge she had been fairly well controlled on diet and 80 units of Lente plus 20 units of crystalline insulin every morning before breakfast. On admission to the Metabolism Ward she was placed on a mixed weighed diet of 2,134 calories containing 88 gm. of protein, 110 gm. of fat and 198 gm. of carbohydrate, and the same insulin dose that she had been receiving at home.

On optimal insulin control the fasting blood sugars ranged from 60 to 200 mg. per cent and the urinary sugar from 1 to 15 gm. per day. Nitrogen balance was slightly positive. The plasma inorganic phosphorus ranged between 4.6 and 6.0 mg. per cent. The fluctuations in blood sugar and inorganic phosphorus were negatively correlated. The balance for inorganic phosphorus was negative. Urinary corticosteroid excretion was in the normal range. Body weight was steady.

The administration of 3 mg. of glucagon daily resulted in a prompt sharp rise in blood and urinary sugar. Increasing the dose of glucagon to 6 mg. daily produced further rises in both blood and urinary sugar levels. During the glucagon period the fasting blood sugar levels ranged between 180 and 300 mg. per cent and the urinary sugars between 12 and 45 gm. per day. Nitrogen balance changed from positive to negative, plasma inorganic phosphorus levels and body weight fell slightly. Inorganic phosphate balance, water balance, blood eosinophiles and urinary corticosteroids did not change.

Discontinuance of glucagon resulted in a marked fall in blood sugar to normal levels but this was followed by irregular waves of hyperglycemia and glycosuria. Nitrogen balance again became positive and the plasma inorganic phosphorus levels began to rise although the individual daily values for phosphorus and nitrogen continued to be reciprocally related to the blood and urinary sugar levels. No change was noted in inorganic phosphate balance, blood eosinophiles, urinary corticosteroid excretion or water balance. Body weight continued to fall slightly.

Reduction in insulin dose from 100 units to between 40 and 60 units daily resulted in marked but irregular rises in blood and urinary sugar. On 40 to 50 units of insulin daily the fasting blood sugar ranged between 300 and 350 mg. per cent and the urinary sugar

between 60 and 110 gm. per day. Nitrogen balance reverted to slightly negative, plasma inorganic phosphorus tended to fall and urinary excretion of phosphorus tended to increase slightly. Body weight continued to fall slightly. There were no appreciable changes in eosinophile or urinary corticosteroids.

In summary, the repeated administration of glucagon in the patient just presented resulted in increased levels of sugar in blood and urine, increased excretion of urinary nitrogen, with reversal of nitrogen balance from positive to negative, and a fall in serum inorganic phosphorus. The increased urinary nitrogen was greater than that which accompanied similar or greater degrees of hyperglycemia and glycosuria with suboptimal insulin. These findings have been observed in other patients, and a report of our studies completed to date will be published soon.

I should like to comment very briefly on the nitrogen and phosphorus response to glucagon. The mechanism of the protein catabolic action of glucagon is not clear. Dr. Salter has stated that it is not mediated by the adrenal glands, although a synergy between adrenocortical secretion and glucagon is postulated. This is interesting, because in some patients, we have observed a stimulation of the adrenal cortex with administration of glucagon as measured by the increased excretion of urinary reducing corticoids. It is noteworthy that the fall in serum phosphorus in response to glucagon was not associated with increased excretion of phosphorus, suggesting a change in equilibrium between circulating phosphorus and tissue phosphorus. This is even more impressive when we consider that the increased excretion of nitrogen would be normally expected to be accompanied by an increased excretion of phosphorus. We believe that the fall in serum phosphorus does not necessarily reflect increased peripheral utilization of glucose but rather a shift to the liver with the increased glycogenolysis. The blood phosphate-glucose relationships in response to glucagon are similar to the inverse relationships between blood glucose and phosphorus which we have observed spontaneously in diabetics, particularly the unstable type, and reported elsewhere. One is tempted to conjecture that glucagon may be a factor in instability. Finally, I would like to call attention to the fact that glucagon has been reported to be synergistic both with insulin and with adrenal corticoids. Perhaps glucagon may be an important link in integrating the actions of these hormones in metabolic homeostasis. In conclusion, one thing is clear, glucagon is a most interesting substance and deserves continued study.

CHAIRMAN BRANDALEONE: We are very grateful to you for coming at the last minute in place of Dr. Joseph J. Hoet of Boston, who was unable to be here. These papers are open for discussion.

GENERAL DISCUSSION

QUESTION: I was very much interested in Dr. Bromer's slides which demonstrated the decrease of metabolism in the depancreatized animals. I wonder whether this is due to the administration of glucagon, or whether that might be an additional finding of increase or decrease of metabolism which might not be attributable to the administration of glucagon. I also would like to know whether this degranulation is reversible when glucagon is discontinued.

DR. SALTER: We don't know whether or not the decrease in acinar-cell granulation is a direct effect of glucagon. It has been suggested that this change is an indirect outcome of the effect of glucagon on protein metabolism but we do not see degranulation of the acinar cells when protein catabolism is produced with cortisone. However, we cannot produce as severe a negative nitrogen balance with cortisone as we can with glucagon. The effect on the granules is reversible. They reappear three to four days after cessation of glucagon treatment.

DR. BENJAMIN JABLONS: I believe Dr. Anderson showed a slide in which a dog was sensitized to the hyperglycemic effect of glucagon by previous administration of Orinase, whereas in one of the last slides in Dr. Izzo's report on Orinase and glucagon in humans, the use of the sulfonylureas appeared to depress the hyperglycemic effect of glucagon. If I interpreted Dr. Anderson's slide correctly, I wonder whether that might not be an explanation for some of the patients who respond poorly to the sulfonylureas and instead of showing a hypoglycemic effect manifest a hyperglycemic effect. Perhaps the explanation can be found along the line that Dr. Anderson indicated.

GEORGE E. ANDERSON, M.D., *(Brooklyn)*: It is very interesting to me that you picked that up, because we have been routinely performing glucagon-sensitivity tests on all patients treated with Orinase. We find that those patients who respond well to Orinase show before the administration of the Orinase poor or practically no glycogenolytic response to glucagon by test.*

During therapy with Orinase, they develop a very marked response, occasionally, as in dogs, going up three or four hundred per cent in their response. After discontinuing the Orinase for several weeks, this re-

* In the fasting state, with the patient in recumbency, a 20-gauge needle is inserted into an antecubital vein and a slow

sponse to glucagon usually disappears.

We have felt that we can gauge the individual who will respond to Orinase by means of his response to glucagon before administration of the Orinase.

As to the difference between my findings and those of Dr. Izzo, we are speaking of two different things. You can't compare potatoes with tomatoes. The findings should be expected to be entirely different. Dr. Izzo has been using tremendous doses of glucagon. We have been using by comparison trace doses of it, and the two premises are not in any sense comparable. I feel that in tremendous doses you can get suppression of the action of any hormone.

QUESTION: I would like to ask Dr. Salter if he did a glycogen analysis on the liver.

DR. SALTER: Dr. Mary Root has reported an increase in liver glycogen after giving glucagon. We have found the same thing but believe it is a compensatory response that occurs after the immediate effects of glucagon have worn off.

In our experiments we find low glycogen levels during chronic glucagon treatment but twenty-four hours after cessation of treatment, the liver glycogen rises to abnormally high levels.

saline drip attached. Six or eight minutes are allowed to elapse for subsidence of any adrenal medullary effect incidental to the needling. Then in a heparinized syringe, blood is withdrawn continuously and evenly over three minutes by stop watch for glucose determinations. Glucagon (20 *mg.*) is then administered by vein in zero time and the saline drip reattached. Ten minutes later venous blood is again collected over a one-minute period. The anticipated rise in blood glucose in this period is 20 ± 5 per cent of the fasting level. Less than this is considered to be a poor response to the glucagon. Many patients show little or no response whatsoever. These individuals seems to do well on Orinase and vice versa.

Therapeutic Use of Per Os Administered Amino Acid Preparations

The well-integrated function of the digestive organs and the consequent slow absorption of the dietary amino acids present a model or pattern which must be considered in relation to therapeutic trends. It has recently become fashionable to administer protein hydrolysates or mixtures of amino acids in lieu of food proteins. This practice is based on the results of some investigators who have been able to keep human subjects in nitrogen equilibrium by feeding such amino acid preparations; however, long-term feeding experiments are still lacking. On the other hand, it has been established that amino acid mixtures cannot be utilized with the same efficiency as equivalent amounts of protein. The chief reason for this decreased utilization seems to be the extremely rapid absorption of free amino acids with consequent flooding of the tissues with excessive amounts of amino acids which cannot be utilized so rapidly and hence must be partially wasted. A further complication arises from the disturbances which result from an increased concentration of amino acids in the intestines and in the blood.[1] We have already emphasized the fact that, in the course of normal protein digestion, amino acids do not accumulate; after feeding protein digests, however, abnormally high concentrations of amino acids in the intestines may produce intestinal distress and diarrhea. Due to the rapid, unregulated absorption, the blood amino acid levels may increase to values which may lead to nausea and vomiting. It is likely that these disturbances are responsible for the serious aversion which the patient develops towards large quantities of these hydrolysates; in any event, after a few days on such a regimen, it often becomes impossible to provide any notable fraction of the daily nitrogen requirement in the form of amino acid preparations.

From the book *Modern Nutrition in Health and Disease* edited by Michael G. Wohl, M.D., and Robert S. Goodhart, M.D. Philadelphia, Lea & Febiger, 1955. Chapter "Digestion, Absorption and Metabolism of Protein" by Ernest Geiger, M.D., Ph.D., pp. 105-06.

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A Sequential Appraisal of Glucagon

Herbert M. Evans, M.D., Berkeley, California*

Glucagon, discovered and named thirty-three years ago,[1] has not yet been unqualifiedly accepted as a hormone. No clinical disorder attributable to deficiency or excess of glucagon has been established. This is perhaps not so serious because, for example, there could be no doubt of the hormonal nature of epinephrine but no syndrome referable to a deficiency of adrenal medullary secretion is known. Furthermore, evidence accumulates that glucagon secretion is increased by hypoglycemia, by insulin, by growth hormone and by adrenocorticotrophic hormone, and a "hyper" picture may be presented by the obesity hyperglycemia syndrome of mice.

It took a considerable time for us to revert to the Murlin name (glucagon), for the best early studies on the substance were those of Sutherland and Cori who called it the "hyperglycemic-glycogenolytic factor" (HGF). Its resemblance to, but difference from, epinephrine was soon established. Glucagon resembles epinephrine in that both stimulate glycogenolysis and both promote transformation of inactive to active phosphorylase. Glucagon differs from epinephrine in that glucagon has *no* effect on blood pressure, heart rate or eosinophile response, and the glucagon glycogenolytic effect is not blocked by adrenergic blocking agents which are capable of blocking epinephrine.[2] Furthermore, unlike epinephrine, glucagon does *not* produce a breakdown of muscle glycogen to lactate or pyruvate.

Chemical and Physico-chemical Properties of Glucagon. A considerable time ago, Sopp[3] showed that glucagon was a protein closely related to insulin. In 1953 and 1954 Staub and his associates[4] completely purified glucagon and began to characterize the molecule extensively. In 1955[5] he continued to characterize the pure substance. Its molecular weight is 4,200. Unlike insulin, it contains no cystine, proline, or isoleucine, but does possess methionine and tryptophane (absent in insulin), and can crystallize in the absence of zinc or other metals (crystalline glucagon has less than 0.01 per cent zinc). Furthermore, its amino-acid composition shows that it can *not* be a decomposition product of insulin.

Site of Origin. Immediately following the discovery of insulin, Macleod and Collip, encountering the brief but significant hyperglycemia, called this paradoxical. It was not found in Abel's crystalline insulin and therefore correctly attributed by Gerling to a contaminant, for which Murlin in 1924 suggested the name glucagon and the possibility that it was a physiologic substance.

Glucagon occurs in commercial insulin, in the perfusate and extracts of normal pancreas and the beta-cell-free pancreas of alloxanized animals and the duct ligated acinae-free pancreas. It is hence an islet cell product not produced by the beta cells and it seemed reasonable to assign its secretion to the alpha islet cells. Success in the chemical destruction of the alpha cells should have settled this but the employment of cobalt as an alpha-cytotoxic substance was unfortunate.

Volk, Lazarus and Goldner[6] showed that extracts of the pancreas of normal or alloxanized dogs, in which alpha cells have "almost entirely" disappeared after cobalt administration, still contain apparently normal quantities of a hyperglycemic principle. Fodden and Read[7] have shown that the pancreas from cobaltous chloride-treated rabbits contains an appreciable quantity of material with hyperglycemic activity, but that Synthalin A treatment leaves little hyperglycemic activity in the pancreas. A significant study of the glucagon content of pancreatic tissue devoid of alpha cells has now been made by Bencosme et al.[8] They showed that extracts of the uncinate process of the dog's pancreas, which contain no A cells, show virtually no hyperglycemic activity even when large aliquots of pancreas were used.

We are not yet clear as to whether extrapancreatic enterochromaffin cells secrete glucagon. Sutherland did not secure glucagon from the stomach of the hog where argentaffin cells are nevertheless present. According to Fodden,[9] these cells are *not* identical with pancreatic alpha cells. The two cells differ in response to several alphacytotoxic substances. It appears that stomach extracts may have an epinephrine-like substance with action like glucagon on liver slices. Finally, Ferner's claim that alpha

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cells hypertrophy with growth hormone and atrophy after hypophysectomy has received no confirmation. Islets and their cells are apparently seldom hypertrophied.

Relations of Glucagon to Insulin and the Anterior Hypophyseal Hormones. Let us next consider the relations of glucagon to insulin and the anterior hypophyseal hormones, as indicating a true physiologic role of glucagon in carbohydrate homeostasis. Pancreatectomized patients and animals need less insulin for regulation though they are less resistant to ketosis. Insulin sensitivity is greater in depancreatized dogs than in dogs treated with alloxan and the depancreatized dog becomes more ketotic than the alloxanized dog when insulin is withheld. The effect of pancreatectomy on insulin requirement is *not* due to faulty absorption of foodstuffs due to the lack of pancreatic enzymes, for in the alloxan diabetic dog ligation of the pancreatic ducts does not diminish the higher requirement for insulin.[10] The cross-circulation experiments of Foa et al.[11] suggest that the pancreas secretes glucagon in response to insulin-induced hypoglycemia. Sherlock,[12] in studying the response of the liver to insulin, has done hepatic vein catheterizations in man. When insulin was administered, hepatic glucose output was decreased at once; later there was more glucose and the blood sugar was restored to normal. An increase in glucose output precedes the increased hepatic blood flow and lactic acid output which occur when epinephrine is secreted. This may be due to glucagon secretion or as a direct hepatic response to hypoglycemia without need for glucagon intervention. Now, Laurence and Stacey[13] have shown that hexamethonium which is supposed to block sympathetic impulses to the adrenal medulla, does not delay the return of the blood glucose to normal after insulin-induced hypoglycemia. Hence, possibly glucagon is involved. It is interesting, in view of Foa's and of Sherlock's work, that MacGrath and Snedecor[14] have shown that in animals chronically treated with insulin the glucagon content of the pancreas is increased.

Growth hormone administration elicits the secretion of glucagon from the pancreas. Bornstein et al.[15] showed that blood from the pancreaticoduodenal vein of growth hormone-treated cats contains a blood sugar-raising substance which is *not* demonstrable in the femoral vein blood from the same animals. With his cross-circulation technic, Foa[16] has obtained similar findings. It seems exceedingly important that Fodden and Read[17] have been able to isolate a hyperglycemic phosphorylase-reactivating substance from canine pancreatic blood. They showed that a threefold increase in this hormone resulted from pretreatment with growth or adrenocorticotrophic hormone.

In Mayer's[18] studies genetically obese mice have increased glucagon in their pancreas and they respond with marked hyperglycemia to growth hormone administration, whereas nonobese litter mates show *no* such responses.

Van Itallie[19] has some remarks on the subject of an insulin *antagonist* (DeDuve, Ferner, etc.). The word antagonism is often too loosely used in biology. Because sympathetic and parasympathetic nerves may have "opposing" effects on smooth muscle, these systems are presently referred to as "mutually antagonistic." Actually, the nerves of these two systems act synergistically. The most generally agreed-to concept of the action of insulin is that it facilitates the entry of glucose into cells. There is evidence that growth hormone is antagonistic to insulin in this respect,[20] but the evidence with glucagon is conflicting. It most certainly appears that glucagon does *not* interfere with the action of insulin in promoting glucose transfer in peripheral cells. In the sense that glucagon raises the blood glucose whereas insulin lowers it, glucagon is an anti-insulin factor. By the same token, ingested carbohydrate would have to be called an anti-insulin substance, and this would reduce the concept of insulin antagonism to nonsense.

Finally, no conclusive evidence at present exists that glucagon has any primary physiologic action other than to stimulate hepatic glycogenolysis.[21] Its *physiologic* role has yet to be established. That there is an actual physiologic, sensitive interplay of output of glucagon and insulin has not yet been established in spite of an immense amount of work, for instance, that by Anderson,[22] who described fluctuations in the glucose content of hepatic venous blood as due to periodic glucagon releases. Pancreatectomized or cobalt or Synthalin-treated dogs must be used to establish this.

Etiology of Diabetes Mellitus. Is there as yet any bearing of our knowledge of glucagon upon concepts of the etiology of diabetes mellitus? It has certainly not been established that the disease diabetes, or that pancreatectomy diabetes or meta-hypophyseal diabetes result from an oversecretion of glucagon.

SUMMARIO IN INTERLINGUA

Estimation Currente del Studios de Glucagon

Es summarisate, super le base de referentias historic, le stato presente del investigation del natura, del origine, e del function del factor hyperglycemic glycogenolytic que es currentemente designate como glucagon.

Glucagon es un proteina relationate a insulina. Illo differe de insulina in certes de su amino-acidos in un tal

maniera que illo non pote esser considerate como un producto del decomposition de insulina.

Le question de qual cellulas pancreatic es le sito de origine de glucagon remane controverse, e il es non ancora clar si o non cellulas enterochromaffin extrapancreatic secerne glucagon.

Es listate varie studios relative al interdependentia functional de glucagon con insulina e hormon de crescentia. Es concludite que al tempore presente nulle primari action physiologic de glucagon es establite excepte su stimulation de glycogenolyse hepatic.

Quanto al rolo possibile de glucagon in le etiologia de diabete mellite, le facto es sublineate que il existe nulle prova que le morbo diabete o le diabete que resulta de pancreatectomia o diabete meta-hypophyseal es effectuate per hypersecretion de glucagon.

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Historical Review of Oral Substitutes for Insulin

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The search for oral antidiabetic agents is as old as our knowledge of diabetes mellitus. The discovery of insulin, rather than putting an end to this search, gave it a new stimulus. As soon as it had been found that insulin was ineffective when given by mouth, attempts were made to modify the hormone and to protect it against acid or tryptic digestion. Until now no effective oral insulin preparation has become available, but this failure only spurred the search for oral substitutes for insulin.

The various folk medicines which had long been used in the treatment of diabetes were now investigated for any active principles which might act like insulin, if they were not identical with insulin.

It appeared likely that a substance with insulin-like activity might be present wherever carbohydrates are broken down or synthesized, not only in the vertebrate organism but also in lower organisms and plants. Insulin provided now the standard by which the antidiabetic activity of such substances could be measured. Although superior in its method of administration, any oral substitute would still have to prove that it is at least equal to insulin both in its metabolic action and its lack of serious side effects. Numerous plants, fruit and roots were extracted as well as yeast and other microorganisms related to carbohydrate metabolism. A few of these extracts seemed to have some hypoglycemic activity. One may remember the glukokinin of yeast, the blueberry extract myrtillin, and the extracts from beans, phaseolon and amellin. None of them, however, withstood the test of careful controls.[1] The hypoglycemic action, where it could be confirmed at all, was usually not greater than that which could be achieved by dietary regimens or the imponderabilia of faith and suggestion; none was as effective as insulin, and most had severe toxic side effects. This is true also for hypoglycin, the recently investigated extract of a Caribbean fruit which induces marked hypoglycemia, but at the same time vomiting and severe liver damage.[2]

With the increasing knowledge of the interplay of insulin with other hormones, of the steps of metabolic breakdown of glucose and glycogen and of the sites of action of insulin, numerous inorganic and organic compounds and hormones were examined for their ability to restore blood sugar homeostasis. The labors of this systematic search might best be exemplified by referring to only one rather recent study in which 249 compounds were screened for their possible use as insulin substitutes.[3] This study included steroids, sulfur containing compounds, nitrogen compounds as amines, amidines, carbamates, urea derivatives, oxygen compounds, alcohols, ethers, esters, carboxylic acids, quinones, dyes, vitamins and heavy metals, as antimony, bismuth, arsenic and mercury. A few substances had suggestive hypoglycemic action, but none seemed worthwhile for further testing as an insulin substitute or as an antidiabetic agent. Likewise, neither the substitution of glucose by other sugars, nor trials with intermediary glucose breakdown products, such as glucose-1-phosphate,[4] yielded practical therapeutic results. It is perhaps too early to ask for therapeutically applicable results from the newer studies on insulinase, enzymatic degradation of insulin and insulin-binding. Yet, it is not unreasonable to expect that the search for inhibitors of the peripheral inactivation or destruction of the hormone will lead to agents which may substitute for exogenous insulin by enhancing the action of endogenous insulin or by delaying its inactivation. Mirsky[5] has presented evidence that the hypoglycemic sulfonylureas belong in the group of insulinase-inhibitors.

The recognition of the importance of sulphur as an essential constituent of the insulin molecule and of the sulfhydryl groups as necessary for many fundamental enzymatic systems led to the discoveries that alloxan diabetes can be prevented or ameliorated by glutathione or by substances which increase the sulfhydryl contents in the tissues, such as thiouracil.[6] Other sulfur com-

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pounds, however, seemed to have the opposite effect, aggravating hyperglycemia and glycosuria, and decreasing glucose tolerance.

While thus the systematic screening procedures were rather unsuccessful, a few chance observations appeared to hold greater promises. Yet, up till today we cannot be certain that a useful oral insulin substitute or antidiabetic agent is at hand.

Synthalin, deca-methylene-diguanidin-dichlorhydrate, was introduced as an oral antidiabetic agent[7] when the hypoglycemic activity of guanidin had been discovered by chance during studies with the parathyroid gland.[8] Its role as an insulin substitute was of only short life. Most of us remember its hepatotoxic and renotoxic side effects which removed it soon from our therapeutic armamentarium. Synthalin has gained new theoretical interest in recent years. It was found to cause selective damage to the alpha cells of the islets of Langerhans.[9] This alphacytotoxic action seems, however, not to be the cause of its hypoglycemic effect. It has been demonstrated that Synthalin lowers the blood sugar also in the depancreatized animal, and alpha cell damage has been found to occur not infrequently secondary to liver damage,[10] such as is induced by Synthalin.

Hypoglycemic activity was found accidentally also in some antihistaminic substances, as for instance, antazoline hydrochloride.[11] Their blood sugar lowering effect could be demonstrated both in the normal and the pancreatectomized animal, and in normal and diabetic man. No side effects were noted but the insulin sparing or substituting effect even in large doses was relatively small, so that the clinical application of this observation seemed limited. Studies with related compounds may, however, be of promise.

Probably the most important chance observation is the discovery of the hypoglycemic sulfonamides. This aroused general interest through the now well-known announcement of German investigators[12, 13, 14] in 1955. Historical justice, however, requires the statement that this was rather a rediscovery. A similar observation had been made in France more than ten years earlier. In 1942, Janbon[15] in Montpellier had used for the treatment of typhoid fever a new sulfonamide, which had been prepared by Vonkennel and Kimmig.[16] The compound was p-amino-benzol-sulfonamide-isopropyl-thiodiazole (IPTD). Several of the rather emaciated patients developed convulsions and a few died. Severe hypoglycemia which was protracted and difficult to control even by repeated glucose injections, was recognized as cause of this syndrome. This was the beginning of extensive and fundamental work by Loubatières, the physiologist at Montpellier. Anyone who now reviews his long series of publications is struck by the mass of information which he had accumulated under the most stringent conditions during the war years of 1942-46, and which has important bearing on our present worldwide evaluation of similar compounds. This literature is scattered throughout French medical journals and has been summarized recently in two papers in the Presse Medicale.[17]

Loubatières started out to confirm the hypoglycemic effect of IPTD in the intact dog, both after oral and intravenous administration of this sulfonamide. He determined the sulfonamide blood level and found that it was proportionate to the degree of hypoglycemia. He found that the drug was not effective in the totally depancreatized, but in the partially depancreatized animal, and likewise not in severe alloxan diabetes, but in the alloxanized animal with mild diabetes. Accompanying the hypoglycemia was decrease of glycosuria and increase of liver glycogen. The hypoglycemia occurred after resection of the vagus nerves as well as after large doses of atropin; neither hypophysectomy nor thyroidectomy nor adrenalectomy prevented it. In an anastomosis experiment in which the duodenopancreatic vein of an IPTD treated dog was connected with the jugular vein of an alloxan diabetic dog, the humoral transmission of the hypoglycemic agent was demonstrated. It also was observed that minimal, systematically ineffective doses still caused hypoglycemia when injected into the pancreatic artery. In the hematoxylin-eosin stain, the pancreatic islets after IPTD treatment appeared rather normal, perhaps somewhat larger, and the islets from alloxanized animals seemed to show signs of regeneration. Loubatières concluded from this evidence that his sulfonamide preparation exerted its hypoglycemic effect by a direct action on the islets of Langerhans, probably by stimulating insulin production or secretion. As early as 1946, he considered the possible clinical therapeutic use of these substances and stated: "It is logical to suspect that such hypoglycemic agents may be useful to some degree, which must be evaluated, in the treatment of certain forms of diabetes mellitus."

Instead, however, of embarking on such therapeutic tests, probably warned by the early fatalities in Janbon's clinic, he began an evaluation of numerous related sulfonamides, both in order to find a less toxic agent and to study the pharmacological action of these compounds. He found hypoglycemic as well as hyperglycemic agents and others which exhibited a biphasic action. He concluded that the thiodiazole nucleus was not essential for the hypoglycemic action, that the butyl derivatives

were most active while the amyls, iso-amyls and propyls were less and the ethyl and methyls either ineffective or hyperglycemic.

Although his work had remained largely unnoticed, there were a few investigators, among them Houssay,[6] who confirmed or extended it. La Barre and Reuse[18] confirmed the anastomosis experiments. Bovet and Dubost[19] screened several other sulfonamides. Chen, Anderson and Maze[20] in this country studied another sulfonamide (2-sulfonamilamide, 5-cyclopropyl, 1-3-4-thiodiazole) which was hypoglycemic in the normal rabbit but hyperglycemic in alloxan diabetes and which after prolonged medication was goiterogenic.

In 1953 Von Holt and his associates demonstrated that IPTD had an alphacytotoxic effect[21] and postulated that its hypoglycemic action was not due to a stimulation of the beta cells but rather to destruction of the alpha cells or inhibition of glucagon. Loubatières confirmed their histological finding but maintained that the main action of IPTD was upon the insulin producing system. When shortly afterwards, the German hypoglycemic sulfonylureas were offered, it was because of Von Holt's observation that it was suggested that they too act via the alpha cells.[12, 13, 14]

These new substances are closely related to IPTD but do not contain the thiodiazole nucleus; the two best known are carbutamide (BZ-55) 1-butyl, 3-sulfanil urea and tolbutamide (D-860 or Orinase), its methylated analogue: 1 butyl-3-p-tolylsulfonylurea. Like IPTD, these substances have hypoglycemic effects only in the presence of functioning islet tissue and are ineffective after pancreatectomy and in severe alloxan diabetes. Like IPTD they appear to have toxic side effects. They appear to differ, however, from IPTD inasmuch as no alphacytotoxic action has as yet been demonstrated with them. Thus it seems unlikely that their hypoglycemic effect is mediated through the alpha cells or through glucagon. It also appears probable that the alphacytotoxic effect of IPTD is not related to its action on blood sugar homeostasis. Loubatières' concept, on the other hand, is not yet proved either. Conclusive evidence is still lacking to indicate whether the histologic changes of the beta-cells represent hyperactivity or exhaustion. Moreover, insulin-stimulation alone does not seem to explain satisfactorily the action of these compounds since they are ineffective in diabetic acidosis, where insulin shows its most significant effect, and since hypophysectomy does not intensify their effect while absence of the pituitary markedly increases insulin sensitivity. Alternate or additional mechanisms of action have therefore been proposed as inhibition of insulinase,[5] delay of degradation of insulin or interference with hepatic glycogenolysis. A discussion of these hypotheses and their experimental evidences, however, does not belong in an historical review. This is the object of present day research about which the subsequent papers of this Symposium will report.

In closing we may ask, whether the discovery of the hypoglycemic sulfonylureas has provided us with the oral antidiabetic agent for which we have searched for so long. Their hypoglycemic action and their ability to abolish or decrease glycosuria can be considered as established. But it still must be determined whether they induce increased glucose-utilization and whether they are free of harmful effects even after prolonged administration. Since it is known already that they are ineffective in the absence of the pancreas and in ketoacidosis, it will have to be determined to what extent they can be used safely as substitutes for insulin. Already they are a most valuable tool for the further investigation of carbohydrate metabolism and its disturbances, but only the future can show whether they can offer also a new modality for the management of diabetes mellitus in addition to the well established means of diet and insulin.

SUMMARIO IN INTERLINGUA

Revista Historic de Substitutos Oral Pro Insulina

Es presentate un breve historia del cerca pro oral drogas antidiabetic, e le discoperta del sulfonylureas hypoglycemic es describite. Es sublineate le importantia del labores del pionero Loubatières. Ben que il es non ancora possibile dicer si o non le sulfonylureas hypoglycemic va esser utile e innocente in le tractamento de diabete mellite (juxta le duo ben-establite modalitates de dieta e insulina), il es a recognoscer que mesmo nunc le compositos in question representa un significativissime instrumento de investigation del metabolismo del hydratos de carbon e de su dysfunctiones

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The Fuel of Muscular Exercise

In a very recent and exhaustive review of the subject, Gemmill[1] has aptly reviewed the situation with regard to the fuel of muscular exercise as follows:

"From the survey of the literature it is obvious that the use of carbohydrate is of primary importance as a fuel for muscular exercise in man. The evidence comes from the slight increase in efficiency on a carbohydrate diet, the prolongation of muscular effort when carbohydrate is ingested, the fall in blood sugar during long-continued muscular exercise and the production of lactate at the beginning of exercise and during severe exercise. The evidence that protein is used during exercise indicates that is it of secondary importance, probably to supply carbohydrate or carbohydrate intermediates. The results of experiments on fat utilization during muscular work have demonstrated that this substance is used indirectly. There is no experimental evidence at the present time for the direct utilization of fat by mammalian muscle. However, the indirect utilization of protein or fat must be an efficient process, since the exclusive feeding of these substances to man does not have a marked effect on muscular efficiency during short periods of exercise."

However, since Gemmill's review was written, some evidence has been brought forward to indicate that fatty acids can be oxidized by the liverless animal[2] and by muscle extracts in vitro.[3, 4] But whether this occurs in intact muscle, or to what extent it occurs in relation to the total caloric expenditure, has not been determined. As a matter of fact, the work on the whole animal was done under resting conditions, so that the peripheral oxidation of fat observed may have no bearing as regards the fuel of muscular exercise. Hence it is still necessary to conclude that carbohydrate is the chief fuel of muscular exercise.

From the book *Modern Nutrition in Health and Disease* edited by Michael G. Wohl, M.D., and Robert S. Goodhart, M.D. Philadelphia, Lea & Febiger, 1955.

Chapter "The Role of Carbohydrates in the Diet" by Samuel Soskin, M.D., and R. Levine, M.D., p. 150.

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The Mechanism of Action of The Sulfonylureas in Diabetes Mellitus

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This field of inquiry dates back to 1942 when a group of French physicians observed profound hypoglycemic states in patients with typhoid fever, given what was then a new sulfonamide, the isopropyl thiodiazole derivative, IPTD.[22] The hypoglycemia was in some instances very severe, leading to a fatal outcome. Soon after this observation was published, Loubatières and his colleagues began an investigation of the mode of action of this drug and its congeners. In a series of studies published in 1944 and 1946 they[27,28,29,30,31] came to the following conclusions:

a. While IPTD produced hypoglycemia in normal animals, (dogs and rabbits), no hypoglycemia was observed when these drugs were administered to depancreatized dogs.[27]

b. However, if the animals were only partially depancreatized, leaving as little as 1/6 to 1/10 of the pancreas, hypoglycemia followed the administration of the drug.[27]

c. Both Bovet[4] and Loubatières[28] established that the side chain of the molecule may be modified without losing hypoglycemic activity, e.g., the isopropyl, butyl, and amyl derivatives are active; but that with further changes, activity was lost.

d. The sulfonamide portion seemed to be essential for the activity.[28]

e. The instillation of the drug into the duct of Wirsung under pressure, or into the pancreatic artery produced hypoglycemia in the peripheral circulation.[30]

From these studies Loubatières tentatively concluded that IPTD was a pancreatotropic material which stimulated the release and/or the production of insulin, as long as normal beta cells in sufficient quantity were available. He added the observation that administration of IPTD raised the respiratory quotient of normal animals after glucose administration, similar to the administration of a small amount of insulin.[30] The conclusion of all these studies was that the mechanism of hypoglycemic activity was the excitation of the beta cell to release insulin, and that the hypoglycemia itself was due to the released insulin.

In 1947 Chen et al. repeated some of the experiments of Loubatières and added the observation that the cyclopropyl derivative, which he used, was inactive in the severely alloxanized animal. This seemed to support the original contention of Loubatières as to mechanism of action. Similar conclusions were reached by La Barre,[26] who added the observation that the drug was active in the adrenalectomized animal. The pancreatotropic action of Loubatières was supported by an additional experiment in which a donor dog receiving the drug was connected by way of the pancreatic vein to the circulation of a recipient alloxanized animal, and a lowering of the blood sugar was obtained in the recipient animal.[29] No echo of these results appeared in the literature of clinical investigation in France during that period.

In 1953 and 1954 Holt[19] in Hamburg used IPTD and Synthalin to produce hypoglycemia in rabbits. The conclusion of Holt and his co-workers was that these drugs lead to degeneration of the alpha cells. Thus a diminished amount of glucagon was released from the pancreas resulting in uncompensated insulin action and therefore hypoglycemia. At this time several new sulfonamides were being tested for their antibacterial potency and the absence of crystalluria. The first one of these was the butylurea derivative of sulfonamide, known originally as BZ-55 and more recently as carbutamide. Hypoglycemia was detected in the experimental animals used for bio-assay of the bacterial potency, and this observation was quickly transferred for clinical investigation. It soon was reported that the administration of carbutamide by mouth would in many instances depress the blood glucose and the glycosuria of certain diabetics — primarily the older group of obese, nonketotic individuals, especially those who had not received

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insulin for any great length of time.[3] The clinicians who were reporting these first results discussed them from the standpoint of the mechanism of action proposed by Holt, namely depression of the alpha cells of the pancreas. It soon became evident that most juvenile diabetics did not respond to the drug.[3] A similar unresponsiveness was seen in severe adult diabetics who had received insulin for many years.[3]

Soon thereafter a modified butylurea derivative was produced which contained a methyl group in place of the p-amino group on the benzene ring. This substance is known as tolbutamide or Orinase.* In the late fall of 1955 both of these drugs became available to American investigators. The experimental and clinical results achieved thus far may be used to determine, with a certain degree of reasonableness, the possible mechanism of action of these drugs.

A substance which reduces the blood sugar could do so, presumably, in many ways:

1. *Insulin-like Action.* Such a drug could lead to a greater utilization of blood glucose in the peripheral tissues, in the same manner as does insulin. In other words, it could be an insulin substitute, and its activity would therefore not depend upon the presence of insulin or any other mediators.

2. *Action on Hepatic Mechanisms for the Release and Manufacture of Glucose.* It could diminish the output of sugar from the liver by inhibiting one of the essential steps needed in the release of glucose from the hepatic cell. Many steps of the glycolytic scheme come to mind as possible points of action, especially the enzyme glucose-6-phosphatase which releases glucose from glucose-6-phosphate.

3. *Insulinase Inhibition.* During the past five or six years studies have appeared concerning the metabolism of insulin. There exists in the liver a proteolytic system which can lead to the degradation of insulin. Whether this proteolytic system is specific for the insulin molecule or is an enzyme which can also attack other protein molecules such as glucagon, *b*-corticotropin, or casein is still under discussion. The system is generally referred to as insulinase.[36] If insulinase plays a physiological role, in the sense that the amount of insulin secreted is subject to proteolytic activity before it passes into the peripheral circulation, then modifications of the activity of insulinase will either increase or decrease the amount of insulin available to the peripheral cells. A drug which lowers blood sugar could do so by inhibiting this proteolytic system, thereby allowing more insulin to reach its effector cells. In this case the sulfonylureas would not be expected to act in the complete absence of insulin, but would be active in the presence of the pancreas or in the presence of exogenously introduced insulin.

4. *Pancreatotropic Effect.* The activity of the drug insofar as hypoglycemia is concerned may be wholly pancreatotropic, as in the original conception of Loubatières. It would therefore not depend either on peripheral activity or liver activity, but would depend on the amount of insulin available in the beta cells. These would be stimulated to release preformed insulin, and such a release might stimulate the further production of the hormone.

5. *Alpha Cell Depression.* The α cell depression hypothesis of Holt implies that insulin and glucagon are normal antagonists, and that a reduction of glucagon secretion would enhance the blood sugar lowering capacity of the normal amount of insulin. In this instance the presence of the pancreas would be imperative for the action of the hypoglycemic drugs.

Which one of these five possible modes of action has the clearest supporting evidence in its favor? It is possible that the drugs involved exert more than one of these possible actions? It is not difficult to throw doubt on some of the hypotheses or to dismiss them altogether for lack of positive evidence. It is far more difficult to arrive at a reasonable conclusion in favor of any single hypothesis. We shall endeavor to present first the negative evidence against some of the suppositions, and then examine what steps are needed to ascertain completely the validity of the proposed mechanisms of action.

In the eviscerated animal, be it the dog [5] or the rabbit,[46] the sulfonylureas have no effect on the amount of glucose which enters the peripheral tissues per unit time. Nor do these drugs affect the rate of the ultimate volume of distribution of nonutilizable sugars which are responsive to insulin.[15] Using diaphragm and adipose tissue it can be shown in vitro that these drugs have no significant activity in respect to increasing the rate of glucose uptake.[25] It is therefore evident that these drugs are not substitutes for insulin and the peripheral mode of action which would operate by increasing glucose utilization has the strongest negative evidence against it and can at this time be dismissed as a serious contender for a mechanism of action.

The α cell depression hypothesis formulated by Holt[19] has little evidence in its favor in view of recent work in which no histological changes of significance in the α cell could be obtained.[9, 45] Histological evidence of

*Orinase®, The Upjohn Company, Kalamazoo, Michigan.

major damage to cells by sulfonylureas has not been confirmed. Recent work by Loubatières[32] again raises the issue of action on the α cell, but from the available data at the moment, it can be concluded that the α cell depression hypothesis cannot account for the rapid hypoglycemia which follows the administration of these drugs.

The possible role of insulinase is of wider significance than the question of the effect of sulfonylureas on the system.[36] It has not yet been definitively shown that this proteolytic system is normally active in regulating the amount of insulin available to the periphery. The evidence for the strict specificity of the proteolytic system for the insulin molecule is not complete.[42] In addition, the amount of carbutamide or tolbutamide which is needed to inhibit insulinase seems too large to correspond with the acute hypoglycemic dosage effective in the normal animal.[48] Some attempts at purification of insulinase have led to a lessened effect of the sulfa drugs on the more purified preparation than on liver homogenates directly.[44] It is quite possible that these drugs as well as drugs similar in structure, whether hypoglycemic or not, will at certain dose levels inhibit many enzyme systems including the insulinase system; but this does not at the moment warrant the conclusion that the mechanism of immediate action is via a depression of insulinase. It is, however, likely that while the acute effects of the drugs may not be explained in terms of insulinase, the chronic administration of these substances and their consequent effects on liver may include a depression of insulinase activity.

There is a body of evidence supporting the thesis that the sulfonylureas may, at certain dose levels and under particular conditions, inhibit glucose output by the liver in vitro[17] as well as in vivo.[34, 38, 42] Several laboratories have reported that in humans not ordinarily responsive to these drugs, one may obtain a response when measuring the rate of fructose to glucose transformation. When fructose is given to an uncontrolled diabetic the blood glucose level rises, due presumably to the additional glucose manufactured from the ingested or injected fructose via a series of transformations in the liver (through the trioses to glucose-6-phosphate and then to free glucose by the action of glucose-6-phosphatase). When the group of diabetics who experience no lowering of the blood glucose in response to the sulfonylureas (juvenile and unstable types) are given a fructose load, the rise in blood glucose is less when they are treated with these drugs than during a control period with no treatment.[34, 38, 42] It was therefore tentatively concluded that one of the actions of these drugs that could account for hypoglycemia is an inhibition of one of the steps leading to the formation of hepatic glucose. This is an extremely important issue to settle because any drug which chronically affects the set of systems in the liver which manufacture and release glucose, would ultimately be expected to produce functional and perhaps anatomical damage to the liver.

The postulate that these drugs act to lower the blood glucose by means of inhibiting liver glucose output does not, however, explain why pancreatic tissue seems necessary for the action to become manifest. It would be expected that in the completely depancreatized animal these drugs should still exert an inhibitory effect on gluconeogenesis and sugar output. Yet this does not happen. In many laboratories, it has been confirmed that in the depancreatized animal[15, 21, 27, 46] the drugs do not by themselves lower the blood glucose, nor do they do so in cases of diabetes mellitus consequent to total pancreatectomy.[12, 16, 34,40] It has also been established that in the vast majority of juvenile diabetics the drugs possess no hypoglycemic activity,[2, 3, 6] except, perhaps very soon after onset.[2, 24] Wrenshall's studies[49] are consonant with such a division of action, since they have shown that the juvenile diabetic possesses a pancreas containing practically no extractable insulin; while the glands of adult diabetics may have amounts of insulin in the pancreas averaging 25 to 30 per cent of normal. Wrenshall's graph of insulin content versus age of onset of diabetes[49] is similar to that relating activity of these drugs in producing hypoglycemia in the diabetic population. The reported actions on liver sugar output may have little to do with the production of acute hypoglycemia under ordinary therapeutic circumstances. Thus, one should examine whether the drugs, in amounts which are needed to control adult diabetics or in the minimal amounts needed to produce acute lowering of the blood sugar in the normal, will depress liver sugar output. Sulfonylureas may not be absolutely specific for action on the beta cell; the beta cell may only be a more sensitive station for the activity of such drugs. In larger amounts it is possible that some functional activities of the hepatic cell and of other cells in the body are affected in addition.

We come then to the best documented of the theories of activity, the original suggestion of Loubatières of the action on the release and/or production of insulin by the pancreas. In addition to the evidence presented by Loubatières up to 1946, in support of the pancreatotropic action of IPTD, there has recently been accumulated additional evidence in support of such an action of the sulfonylureas. It has been confirmed that, on

the whole, the sulfonylureas are effective in the adult diabetic whose pancreas presumably contains an adequate amount of insulin,[2, 3, 10] but the drugs are almost completely ineffective in the juvenile diabetic who has little or no insulin in his glands.[49] The drugs have been tested in patients whose diabetes followed pancreatectomy for carcinoma[12, 16] and persistent hypoglycemia.[16, 40] In such instances, there is no evidence of a hypoglycemic action; neither does there seem to be significant potentiation of exogenous insulin. It has been shown in humans[12, 42] and in experimental animals[21] that the other endocrine glands which play a significant role in carbohydrate metabolism are not necessary for the action of the sulfonylureas. Thus the drugs are active in hypopituitarism, experimental[21] or clinical,[12, 16, 42] in Addison's[12, 16] disease, and in the presence of thyrotoxicosis.[39]

Colwell[8] and his co-workers have tested the effects of the drugs in dogs, when given by infusion into the pancreatic artery. It has been established that the sulfonylureas are more effective when given by this route and doses which would be ineffective if given via a peripheral vein, evoke a distinct hypoglycemic response when injected directly into the pancreatic artery.

The problem of potentiation of exogenous insulin by these drugs is as yet unresolved. In most instances, there has been no evidence that the insulin sensitivity curve in man or animals changes after the administration of the sulfonylureas.[12, 39, 42] However, Houssay has demonstrated that one can get potentiation of insulin action in the depancreatized dog by the administration of very large amounts of the sulfonylureas.[20] In amounts which are normally hypoglycemic insulin potentiation does not seem to occur.

In order to resolve the issue of whether the hypoglycemic activity of the sulfonylureas depends upon the inhibition of some liver factor, be it one concerned with insulin metabolism or with glucose output by that organ, hepatectomy was performed, leaving the circulation of the pancreas intact, but emptying into the vena cava. The difficulty, aside from the technical problems of surgery, is the fact that the hepatectomized animal needs a constant injection of sugar in order to maintain the blood sugar level; and it is difficult to judge small degrees of hypoglycemia. Some preliminary experiments from this laboratory suggest that the sulfonylureas will depress the blood sugar in the presence of the pancreas even in the absence of the liver. Dulin[11] has performed hepatectomy experiments in rats and has found that tolbutamide given intravenously to hepatectomized rats, which were maintained by the infusion of glucose, did produce a very significant drop in the blood sugar. Since work from this laboratory[15] and that of Wick[46] has indicated that neither tolbutamide nor carbutamide would depress the blood glucose in the absence of both the pancreas and the liver, there is only one conclusion that one can derive from the experiments on hepatectomized animals: The liver is *not necessary* for the hypoglycemic action of the sulfonylureas. However, the pancreas seems to be necessary. One cannot conclude from these experiments that in the intact animal the liver does not participate in the hypoglycemia after sulfonamide administration; one can only conclude that the organ is not essential for the expression of the blood sugar lowering capacity. It may be that the sulfonylureas excite the secretion of insulin from the β cell, and the hypoglycemia consequent to the release of a small amount of insulin is enhanced by an additional action of the drugs on the inhibition of liver sugar output or the inhibition of insulin degradation. However, one may safely conclude that in the absence of any positive evidence that the liver plays an essential role in the lowering of the blood sugar, that the first and more specific attack of the sulfonylureas is on the β cell.

If the hypoglycemic effect of the sulfonylureas is due ordinarily, at the low dosage level, to the stimulation of the β cell and the release of insulin from it, then it is argued that the phenomena which follow the lowering of the blood sugar by insulin should also follow the lowering of the blood sugar by the sulfonylureas. Glucose tolerance should be improved and there should be an increase in glucose breakdown to CO_2 and in the deposition of muscle glycogen; the inorganic phosphate in the blood should fall, et cetera. Efforts to demonstrate these phenomena have yielded a large mass of information, but the results have not been uniform.

Thus Elrick and Purnell[41] report that after tolbutamide administration the hypoglycemia was not accompanied by an increase in the A-V difference for sugar in the limb vessels. However, Goetz,[16] using essentially the same technics, observed the same increase in A-V difference as after a moderate dose of insulin. Improvement of glucose tolerance has been reported and has also been denied.[12, 42, 1] The initial blood glucose is lowered but in many instances the tolerance curve remains the same. In many experiments insufficient time was given for the action of the sulfonylureas. The increase in labeled CO_2 from isotopic glucose has been reported[35] and also denied.[34, 46] An increased formation

of muscle glycogen from isotopic glucose has recently been reported by Miller[35] and by Frawley.[14] It may be seen therefore that this problem is not completely resolved, and it seems to us that before any definitive judgment is possible, that comparisons must be made between the sulfonylureas and amounts of insulin which approximate those likely to be released from the gland. Most comparisons have been made using a single comparatively large dose of insulin.

In 1955 Loubatières returned to the work on sulfonamide derivatives and showed that in partially alloxanized rabbits treatment with IPTD and other sulfonamide derivatives tended to restore pancreatic function to normal in a much shorter period of time than would have occurred spontaneously.[33] The glycosuria and hyperglycemia reverted to normal in four to five days instead of the usual four to six weeks. Histological examination tended to show some regenerative tendency in the islets, presumably under the influence of the sulfonamide derivatives.[32] There is a suggestion from these experiments that in addition to stimulating the beta cell to release stored insulin, the drugs may actually stimulate the growth of cells that are not yet irreversibly damaged. Whether such a situation can ever be found in the human diabetic is doubtful at the moment, but it again supports the notion that one of the more specific actions of these drugs is exerted on the islet cells, especially of the β variety.

CONCLUSIONS

Various hypotheses concerning the mechanism whereby certain sulfonylurea compounds lower the blood glucose level have been examined. The evidence gathered since 1942 in both man and experimental animals favors the view that these drugs are able to stimulate the β cells of the islets of Langerhans to secrete stored insulin and perhaps also to produce more of the hormone. In addition, the sulfonylureas may under certain conditions affect liver cell activity, either by inhibiting in some manner the output of sugar or by reducing the activity of an insulinase system.

A. In favor of the pancreatic site of action are:

1. Ineffectiveness in pancreatectomized animals and humans.
2. Ineffectiveness in eviscerated animals.
3. Cross-circulation data evidencing presence of insulin-like substance.
4. Hypoglycemic response in hepatectomized animals.
5. Correlation of effectiveness in humans with insulin content of pancreas.
6. Hypoglycemic response with small doses injected into the pancreatic artery.
7. Histologic changes in β cells suggesting trophic or stimulating effect.
8. Ineffectiveness in completely alloxanized animals with definite hypoglycemic response in partially alloxanized animals.
9. Ineffectiveness in juvenile diabetics, and in some unstable adult diabetics.
10. Demonstration of some of the metabolic phenomena known to follow the injection of insulin.

B. Evidence tending to suggest an additional hepatic effect:

1. Difference in rate of fructose to glucose transformation under influence of the drugs.
2. In vitro studies indicating decreased glucose-6-phosphatase activity following treatment with these drugs.
3. Failure to demonstrate in all instances metabolic phenomena known to follow the injection of insulin.
4. An insulin potentiating effect in depancreatized animals given large doses of the drugs.
5. In view of the fact that the action of the sulfonylureas becomes evident generally in the presence of functioning β cells or of exogenous insulin, it would seem at present that an influence on hepatic insulinase is more probable than a direct action on the enzymes of glucose production.

SUMMARIO IN INTERLINGUA

Le Mechanismo del Action del Sulfonylureas in Diabete Mellite

Esseve examinate plure hypotheses in re le mechanismo per que certe compositos de sulfonylurea reduce le nivello de glucosa in le sanguine. Le informationes colligite depost 1942 ab humanos e ab animales experimental supporta le these que iste drogas es capace a stimular le cellulas beta del insulas de Langerhans a secerner insulina de reserva e forsan etiam a producer quantitates additional de ille hormon. In plus, le sulfonylureas affice sub certe conditiones le activitate del cellulas hepatic. Isto occurre in un de duo manieras: Per inhibir in le un o le altere maniera le rendimiento de sucro o per reducer le activitate de un systema de insulinase.

A. Le argumentos in favor del sito pancreatic del action es:

1. Inefficacia in pancreatectomisate animales e humanos.
2. Inefficacia in eviscerate animales.
3. Presentia de un substantia insulinoide rendite evidente in observationes de circulation cruciate.

4. Responsa hypoglycemic in hepatectomisate animales.

5. In humanos, correlation del efficacia con le contento de insulina in le pancreas.

6. Responsa hypoglycemic post parve doses injicite in le arteria pancreatic.

7. Alterationes histologic del cellulas beta que pare signalar un effecto trophic o stimulatori.

8. Inefficacia in animales completemente alloxanisate e presentia de un definite responsa hypoglycemic in animales partialmente alloxanisate.

9. Inefficacia in diabeticos juvenil e in certe adultos instabile.

10. Demonstration de certe phenomenos metabolic que seque cognoscitemente le injection de insulina.

B. Observationes que pare suggerer in plus un effecto hepatic:

1. Differentia del proratas del transformation de fructosa in glucosa sub le influentia del drogas.

2. Studios in vitro que indica un reduction del activitate de glucosa-6-phosphatase post tractamento con le drogas.

3. Non-successo in demonstrar in omne casos phenomenos metabolic que seque cognoscitemente le injection de insulina.

4. Un effecto de potentiation de insulina in animales pancreatectomisate quando illos recipe grande doses del drogas.

5. Viste le facto que le action del sulfonylureas deveni generalmente evidente in le presentia de functionante cellulas beta o de insulina exogene, il pare al tempore presente que un influentia super insulinase hepatic es plus probabile que un action directe super le enzymas del production de gluocsa.

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Testosterone and Nitrogen Retention

Albright tried to establish a correlation between nitrogen retention and increase in body weight. But such correlation was found in animals only for the first few days of nitrogen retention; then the body weight showed a "wearing off" effect. But even in such cases testosterone does not seem to have a general anabolic effect; it particularly influences as could be expected the growth of the accessory sexual tissues which accounts for about 50 per cent of the weight increase.

Another argument to prove that the nitrogen retained after testosterone is actually used for body protein synthesis was that the ratio of retained nitrogen to the retained potassium was similar to the ratio present in normal muscle tissue, i.e., 2.5 to 3.5. This argument, however, does not agree too well with actual observations in convalescent surgical patients who retain potassium at a ratio of 5 mEq. upwards per gram nitrogen.

A further important factor to be considered is that testosterone injection often leads to retention of large amounts of water which results in more substantial increases of body weight than calculated from the N-retention.[1]

From the book *Modern Nutrition in Health and Disease* edited by Michael G. Wohl, M.D., and Robert S. Goodhart, M.D. Philadelphia, Lea & Febiger, 1955. Chapter "Digestion, Absorption and Metabolism of Protein" by Ernest Geiger, M.D., Ph.D., pp. 134-35.

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Studies on the Actions of Oral Hypoglycemic Compounds

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The arylsulfonylureas are effective hypoglycemic agents in certain diabetics. A number of possibilities as to their mode of action have been investigated in our laboratories and clinics. The present paper is principally an abstract of studies that have been reported in detail elsewhere.

To evaluate the hypothesis that sulfonylureas act as pancreatic alpha cell toxins,[1] with subsequent diminution of glucagon secretion, Cox and others[2] fed young rats tolbutamide and carbutamide for twenty-seven days. Neither growth changes nor histological alterations in sections of liver, kidneys and pancreas were detected. The pancreatic alpha and beta cells appeared normal. Tyberghein and Williams[3] have performed experiments to determine alterations in serum glycogenolytic activity (presumably glucagon) in animals fed hypoglycemic doses of sulfonylureas, but final comment as to the results of this work must be withheld at present. The lack of structural change of the alpha cell and the observations of others[4] that these substances are ineffective in severely alloxanized animals make the alpha cell toxicity hypothesis seem unlikely.

Most investigators agree that sulfonylureas are ineffective in lowering blood sugar in pancreatectomized subjects. We were privileged to observe the effect of carbutamide on a totally pancreatectomized patient.[5] No hypoglycemic effect was noted, and further, his insulin requirement was not lowered when the oral drug and insulin were administered simultaneously.

Intravenous glucose-insulin, glucagon and epinephrine tolerance tests were performed on six patients before and during successful oral antidiabetic therapy.[5] A given amount of insulin was no more effective during than prior to therapy and suggests that sulfonylureas do not increase the action of insulin per se. The hyperglycemic effect of glucagon or epinephrine was not altered by the oral drugs in therapeutic doses, tending to indicate that sulfonylureas do not act by inhibiting the reactivation of phosphorylase in liver and/or muscle. One would expect to see at least a quantitative decrease in the degree of hyperglycemic response if this were the case.

To try to localize the action site of these compounds more precisely, a series of experiments were carried out in adult female rats by giving sodium tolbutamide intravenously and determining the percentage blood sugar fall in one hour.[5] Intact, nephrectomized, hepatectomized, hypophysectomized, and adrenalectomized rats were used. In figure 1, the net percentage blood sugar fall is indicated by the solid bars and was determined by subtracting that fall occurring in control animals from the percentage decrease produced by

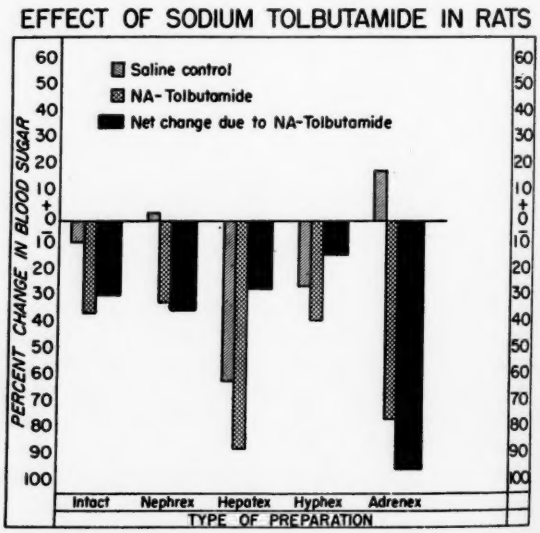

FIG. 1. Neither nephrectomy, hepatectomy, nor hypophysectomy alters the hypoglycemic response to sodium tolbutamide, whereas an increased effect is noted in adrenalectomized preparations. (Modified from a figure appearing in Diabetes, Sept.-Oct., 1956.)

Presented at the Symposium on Insulin, Glucagon and the Oral Hypoglycemic Sulfonylureas sponsored by The Clinical Society of the New York Diabetes Association, Inc., on Oct. 12, 1956.

From the Department of Medicine, University of Washington School of Medicine, Seattle 5, Washington.

tolbutamide. No difference in response was noted between intact, nephrectomized, and hypophysectomized animals. Adrenalectomized preparations had a greater percentage fall. The hepatectomized rats, which were also gastrointestinally eviscerated, showed the same degree of response as did the intact animals. As these were acute experiments and circulating insulin was still present, this might be construed as evidence against the hypothesis of Loubatières,[6] that sulfonylureas act solely by increasing insulin secretion. This response did suggest that sulfonylureas were insulin-degrading enzyme inhibitors in vivo. If this were so, then removal of the liver, which is a major source of these enzymes, would allow a greater inhibition of the enzyme remaining in other tissues. The circulating insulin present would then be degraded less rapidly and a hypoglycemic response would occur.

Both tolbutamide and carbutamide are inhibitors of so-called "insulinase" in in vitro systems when concentrations of about 1×10^{-2} molar are used. To determine the biological significance of this observation Williams and Tucker[7] devised the experiment illustrated in figure 2. Insulin was incubated with buffer for one hour and then an aliquot injected intraperitoneally into mice and a marked fall in blood sugar resulted in 30 minutes (combination I). Repeating this procedure but adding tolbutamide either before or after incubation of insulin did not significantly alter this response (combination II, VI). The addition of insulin degrading enzyme to the initial combination resulted in complete loss of hypoglycemic potency (combination III). However, if tolbutamide is added to this last combination (III) an appreciable effect remained, indication that a marked sparing of insulin degradation occurred (combination IV). When tolbutamide is added after incubation, however, no such effect was noted (combination V). It must be emphasized that the concentrations of drug used in these studies were considerably greater than that present in animal tissues following a hypoglycemic dose of sulfonylurea and when concentrations of drug in this magnitude are used in in vitro studies, no inhibition can be detected.

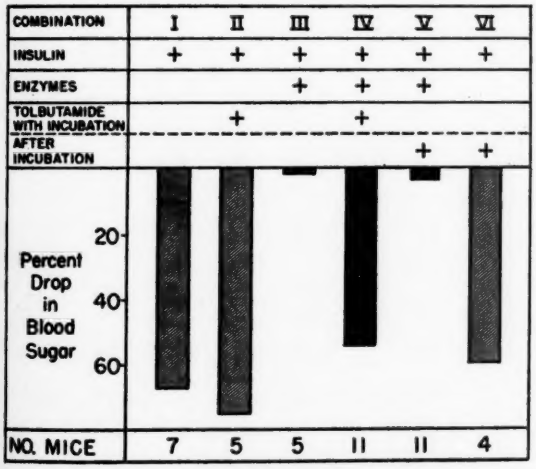

FIG. 2. Tolbutamide is an effective inhibitor of insulin-degrading enzyme under certain experimental conditions where high concentrations of the compound are present. (This figure appeared in Metabolism, November, 1956.)

As a corollary to the enzyme inhibitor studies, the influence of sodium tolbutamide on insulin-I^{131} distribution and degradation in rats was investigated.[2] Intact adult animals were given a tracer dose of insulin-I^{131} intravenously, in the presence of hypoglycemic amounts of the sulfonylurea. The tissue distribution and degradation of the hormone were determined fifteen minutes later and compared to those in animals who had not received the tolbutamide. No differences in plasma, liver, kidney, or muscle concentrations were noted in intact animals, This observation seemed to cast further doubt on the insulin degrading enzyme inhibitor concept as a principal mode of action. If tolbutamide produced hypoglycemia by this means, one would expect to see a smaller amount of degraded material present in the body, but this was not the case.

If sulfonylureas impaired the hepatic release of glucose this would help explain the observed effects of these substances on maturity-onset diabetes. Figure 3 shows experimental work by Tyberghein and others[8] illustrating that while blood glucose falls, liver glycogen changes little in fasted rats who received 800 mg. per kg. of the drug orally in divided doses during a twenty-four hour fast period, as compared to rats who did not receive tolbutamide. A glucose gavage of 500 mg. given twice during the same period abolishes the liver glycogen difference while the hypoglycemic effect remains. These observations suggest that while the conversion of glucose to glycogen is not interfered with, the hepatic release of glucose is quantitatively aborted. A logical site for this reaction to occur is at the glucose-6-phosphate to glucose step in intermediary metabolism, which is catalyzed by a specific phosphatase. If sulfonylureas inhibited glucose-6-phosphatase activity, hypoglycemia might occur.

To test this hypothesis Tyberghein and associates[8] performed assays of glucose-6-phosphatase activity in rat livers. These animals were fasted for twenty-four

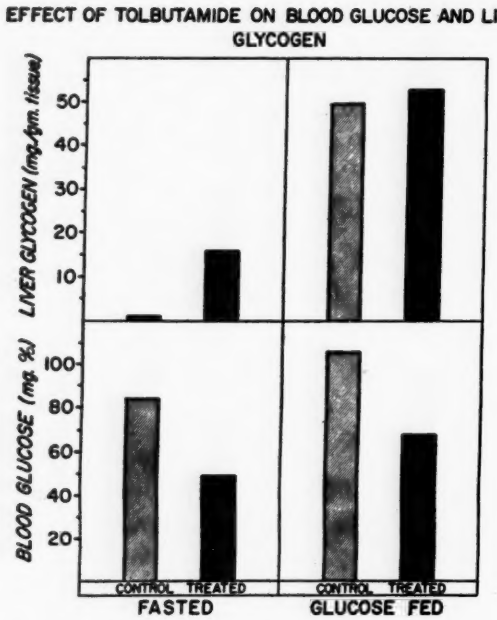

FIG. 3. The hepatic release of glucose is decreased in rats given hypoglycemic doses of tolbutamide.

hours and given tolbutamide as in the previous experiment. After sacrifice, liver homogenates were prepared and incubated with glucose-6-phosphate for thirty minutes. Figure 4 illustrates that a 19 per cent decrease in the enzymatic activity occurred as compared to the controls considering the liberation of inorganic phosphate as an index of enzymatic activity. The respective standard errors top the columns. Whether this alteration is a direct effect of sulfonylurea therapy

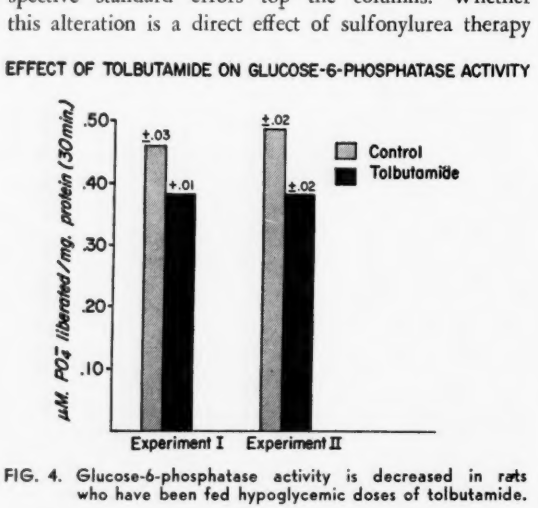

FIG. 4. Glucose-6-phosphatase activity is decreased in rats who have been fed hypoglycemic doses of tolbutamide.

or a reflection of increased insulin or other activity cannot be stated as others[9] have shown that insulin treatment also decreased glucose-6-phosphatase activity.

SUMMARY

1. This series of studies has demonstrated that sulfonylureas do not change the effectiveness of a given amount of exogenous insulin, glucagon or epinephrine, and do not alter the distribution and degradation of insulin-I^{131}. The compounds are not hypoglycemic in the absence of the pancreas but are fully effective in the absence of the kidneys, pituitary and adrenals.

2. Carbutamide and tolbutamide are effective inhibitors of the enzymatic degradation of insulin in vitro which may explain why tolbutamide is hypoglycemic in acutely hepatectomized rats. It is unlikely, however, that this action accounts for the hypoglycemia in the usual in vivo situation.

3. Experimental evidence has been presented to show that tolbutamide interferes with the release of glucose by the liver, perhaps by decreasing glucose-6-phosphatase activity. Other investigators have made similar observations and in addition have obtained evidence to show that other enzymatically regulated reactions in the liver are altered in the presence of sulfonylureas. Whether or not these compounds are effective in the absence of the liver is a debatable point.

4. Although many effects of the sulfonylureas can be demonstrated under special conditions, no one of them can be singled out as *the* mode of action. However, it seems most likely that the liver is a *major site* of action of these compounds.

SUMMARIO IN INTERLINGUA

Studios in Re le Actiones de Compositos Hypoglycemic Administrate per Via Oral

1. Iste serie de studios ha demonstrate que sulfonylureas non altera le efficacia de un date quantitate de exogene insulina, glucagon, o epinephrina e etiam que illos non altera le distribution e le degradation de insulina a I^{131}. Le compositos non es hypoglycemic in le absentia del pancreas, sed lor efficacia remane intacte in le absentia del renes e del glandulas pituitari e adrenal.

2. Carbutamido e tolbutamido es efficace inhibitores del degradation enzymatic de insulina in vitro. Isto explica possibilemente proque tolbutamido es hypoglycemic in acutemente hepatectomisate rattos. Tamen, il non es probabile que iste action explica le hypoglycemia in le usual situation in vivo.

3. Es presentate datos experimental que indica que

tolbutamido obstrue le liberation de glucosa per le hepate, possibilemente per reducer le activitate de glucosa-6-phosphatase. Altere investigatores ha facite simile observationes e in plus ha obtenite datos que supporta le conclusion que altere reactiones a regulation enzymatic in le hepate es alterate in le presentia de sulfonylureas. Si o non iste compositos es efficace in le absentia del hepate remane un puncto controverse.

4. Ben que multe effectos del sulfonylureas pote esser demonstrate sub conditiones special, nulle effecto individual pote esser distinguite como le specific modo de action. Nonobstante, il pare multo probabile que le hepate es le sito major del action de iste compositos.

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"Why Do Animals Eat?"

The progress which obviously has marked the history of the science of nutrition during the past fifty years sometimes causes us to overlook the fact that one of the fundamental and most important problems of nutrition has not been solved. It has not even been studied extensively. It is the question: "Why do animals eat?"—a question of interest to physicians because it touches on the treatment of obesity, leanness and certain metabolic diseases, and the management of feeding problems in infants and children. The answer to this question should explain both overeating and failure to eat, why certain foods are preferred to others, and why particular foodstuffs are sometimes in special demand. It should account for the normal cycles of appetite and satiety, and identify the factors determining the total amount of food consumed per day, the intake at any given meal, and the frequency of mealtimes or feeding periods.

All of these problems appear to be related to the reactions known as hunger, appetite and satiety with which everyone is more or less familiar from personal experience. Since these reactions and the names used to identify them are so widely known, we might expect the nature of the reactions and the meanings of the terms to be equally well known, but this is not the case. In spite of the attempts of numerous authors to define them precisely so that they may be used in a technical sense without misunderstanding, there remains no agreement as to the meaning of any of them. Much of the difficulty comes from the subjective nature of these reactions; they are sensations or psychological states known to us *only* from our own experience. This is a fatal limitation in any attempt to use the words objectively, and it makes it impossible to deal with hunger, appetite, or satiety in any satisfactory quantitative way. They cannot be studied in lower animals, nor in infants. Because of all of these difficulties and others, the attention of physiologists has shifted to a somewhat broader topic, the regulation of food intake, which is objective in its meaning and which refers to a variable—feeding—that can be measured. The word "regulation" is used as it is in speaking of the regulation of other processes in living systems, such as the respiratory minute volume, the blood pressure, heart rate, body temperature, or the concentration of water in body fluids.

From the book *Modern Nutrition in Health and Disease* edited by Michael G. Wohl, M.D., and Robert S. Goodhart, M.D. Philadelphia, Lea & Febiger, 1955. Chapter "Physiology of Hunger, Appetite and Satiety" by John R. Brobeck, M.D., p. 90.

Some Remarks on the Mechanism of Action of the Sulfonylureas

Solomon A. Berson, M.D., and Rosalyn S. Yalow, Ph.D., New York

The three preceding papers we have just been privileged to hear have covered, so thoroughly and elegantly, the past history and present status of the sulfonylureas that there would seem to be little room left for further contributions by the discussants. However, there is always some room for a difference of opinion. Therefore let us take stock of some of the proposed mechanisms of action of these agents for which experimental evidence of one sort or another has been presented.

In the simplest terms, as Claude Bernard first pointed out, the level of blood sugar in a fasting animal is determined by the balance between the rate of glucose supply from the liver and the rate of glucose removal into the tissues (figure 1). The effect of any hypoglycemic agent, therefore, must be due, directly or indirectly, to an acceleration of the rate of removal into the tissues, a diminution in the rate of supply from the liver, or both. The rate of removal is known to be under the influence of insulin, which aids in the penetration of glucose into tissue cells, and it has been suggested that the sulfonylureas exert their hypoglycemic effect through stimulation of insulin secretion,[1] inhibition of insulin degradation[2] or enhancement of the action of insulin peripherally.

The other main possible sphere of activity is the inhibition of glucose release from the liver. Thus, it has been suggested that the sulfonylureas act by diminishing the response to epinephrine or glucagon (perhaps through an effect on the phosphokinase system),[3] or by inhibition of glucose-6-phosphatase[4] or phosphoglucomutase activity.[3]

Other possible actions of hypoglycemic agents might involve the acceleration of glucagon degradation or the inhibition of glucagon secretion. In fact, it has been claimed that the sulfonylureas induce atrophy of the islet alpha cells.[5] However, these observations have not been confirmed by others[6] and, in any event, the *acute* hypoglycemia produced by the sulfonylureas would be difficult to explain on this basis. Still other reports have presented evidence for a retardation of glucose absorption from the gastrointestinal tract[7] and a rather marked inhibition of cytochrome oxidase in the liver slice.[8]

Let us attempt to narrow down the field of possibilities and to integrate some of the apparently diverse actions of these drugs. Earlier this year Dr. Martha Vaughan presented evidence[3] against the theory that inhibition of insulinase is responsible for the hypoglycemic action of the sulfonylureas and from what we have heard today, it appears that Doctors Cox and Levine share this opinion. Together with Doctors Weisenfeld, Goldner and Volk, we have obtained data on the rate of degradation of I^{131} labeled insulin in vivo in rabbits which likewise showed no significant differences between sulfonylurea-treated and control rabbits in the majority of cases.[9] Also in the rat liver homogenate system in vitro, at low or high insulin concentrations, there was no inhibition observed with Orinase in concentrations up to 1.67 mg./ml.[9] At 2.5 mg. Orinase per ml., inhibition was evident and became progressively more marked with increase in Orinase concentration. It must be empasized that the lowest inhibitory concentration observed was ten times as high as the peak plasma concentration obtained in vivo with effective doses. It has also been pointed out by others that the concentration of sulfonylureas in the liver does not exceed that in the plasma.[10, 11] Furthermore, at high concentrations of Orinase in vitro, there is inhibition not only of liver insulinase but of liver adrenocorticotropinase and liver glucagonase as well.[9] Yet the rate of metabolic degradation of I^{131} labeled glucagon in vivo in rabbits is not altered with effective dose levels of Orinase.[9] Nor is the hyperglycemic response to glucagon diminished in the Orinase treated animals.[9]

It has previously been pointed out[9] that the inhibition of a wide variety of enzymes at high concentrations of sulfonylureas in vitro is strongly suggestive of a nonspecific enzyme-inhibitory activity and, therefore, the demonstration of any particular anti-enzyme effect in

Presented at the Symposium on Insulin, Glucagon and the Oral Hypoglycemic Sulfonylureas sponsored by The Clinical Society of the New York Diabetes Association, Inc., on Oct. 12, 1956.

From the Radioisotope Service of the Veterans Administration Hospital, Bronx, New York.

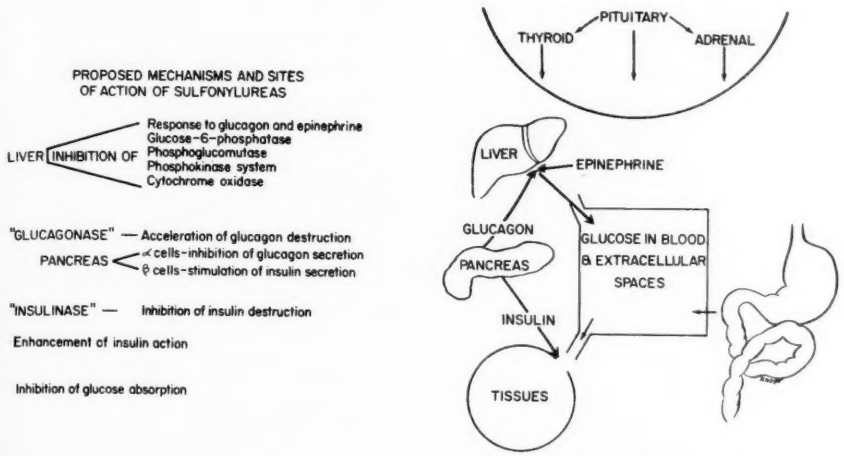

FIG. 1. Simplified model of factors affecting blood sugar concentration and proposed mechanisms for action of sulfonylureas.

vitro is not sufficient in itself to explain the hypoglycemic action of these drugs.

Because of the reported ineffectiveness of the sulfonylureas in the alloxan-diabetic animal,[12] a high index of suspicion has been directed toward some mechanism involving insulin—if not the inhibition of insulin degradation, then perhaps the stimulation of insulin secretion or the enhancement of the peripheral action of the insulin. If this were so, we might anticipate that the sulfonylureas would produce hypoglycemia in the same fashion as does insulin, that is, through acceleration of the rate of removal of blood sugar into the tissues, rather than by a diminution in the rate of release of glucose from the liver. In order to differentiate between these two possibilities, C^{14} labeled glucose was administered intravenously to normal intact rabbits. Mixing of the labeled glucose in its volume of distribution appeared to be complete within about twenty to thirty minutes after which the plasma concentration of the C^{14} labeled glucose decreased exponentially as a function of time (figures 2, 3). Following intravenous administration of insulin (1 unit/kg.), a rapid increase in the rate of removal of the C^{14} labeled glucose from the blood accompanied the almost immediate decrease in blood sugar. The almost identical rate of decline in concentration of unlabeled glucose indicates that insulin also effectively inhibits release of glucose from the liver. However, the fall in blood sugar induced by the intravenous administration of Orinase sodium (12 mg./kg.) required an hour or more to become evident and was not accompanied by any acceleration in the rate of disappearance of C^{14} labeled glucose from the plasma. These findings were confirmed by experiments in which insulin was administered to Orinase-treated rabbits following partial recovery of the blood sugar from the Orinase effect. Here too, in the same rabbits in which Orinase was ineffective in accelerating the rate of removal of C^{14} labeled glucose from the blood stream, insulin did produce an accelerated removal concomitant with the hypoglycemia (figure 3). In one Orinase-treated rabbit there actually appeared to be some retardation in the rate in which C^{14} labeled glucose left the blood stream (figure 3). In line with other evidence regarding enzyme inhibitory activity of the sulfonylureas, this effect could conceivably result from a sulfonylurea-induced inhibition of enzymatic mechanisms concerned with transfer of glucose intracellularly.

We interpret these observations to indicate that the sulfonylureas are capable of producing hypoglycemia without increasing the rate of removal of glucose from the blood stream and are led to the conclusion that in the intact normal rabbit, at least, these drugs exert their hypoglycemic effects independently of the action of insulin but rather through inhibition of release of glucose from the liver. The precise mode of action of the sulfonylurea derivatives on glycogenolysis in the liver has not as yet been defined. It has been demonstrated today that the response to epinephrine and to glucagon is not altered by the sulfonylureas and it has been demonstrated elsewhere[3, 11] that inhibition of glucose-6-phosphatase activity (with the concentrations obtained following effective doses of the sulfonylureas) can likewise not explain the sulfonylurea effect. Thus, few of the originally postulated mechanisms remain for

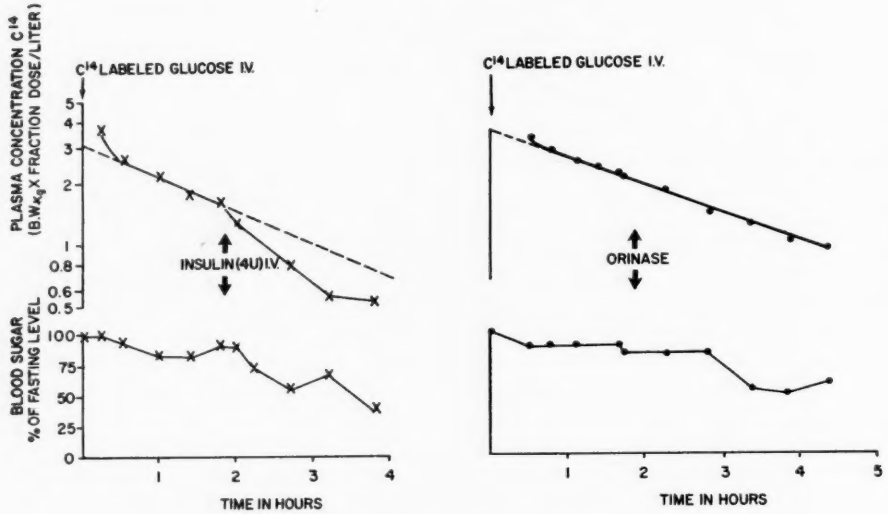

FIG. 2. The influence of intravenous administration of insulin (1 U/kg.) and Orinase sodium (125 mg./kg.) on the rate of disappearance of C^{14} labeled glucose from the plasma.

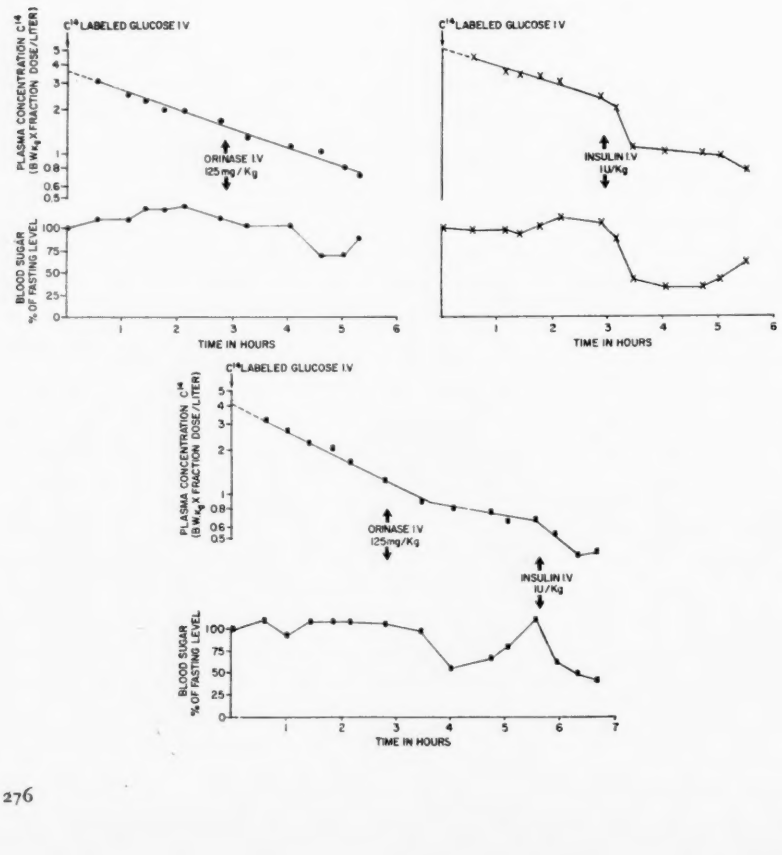

FIG. 3. The influence of intravenous administration of insulin. (1 U/kg.) and Orinase sodium (125 mg./kg.) on the rate of disappearance of C^{14} labeled glucose from the plasma.

serious consideration; but, judging from the intense interest which these agents have aroused, others will undoubtedly be forthcoming shortly.

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General Discussion

RACHMIEL LEVINE, M.D., (*Chicago*): I want to comment here immediately after Dr. Berson's discussion, because there is a very important point of disagreement and one that should be discussed. I wonder, Dr. Berson, whether your findings in the intact rabbit really exclude participation of insulin. If these drugs in the acute experiment act in some manner to inhibit glucose output of the liver, it is very difficult for me to see why they cannot do so in the absence of the beta cells of the islets of Langerhans.

There have been other attempts to test whether insulin is involved in sulfonylurea action. Elrick determined the A-V glucose difference. After insulin this became wider, but after the sulfonylureas it did not. Opposed to these negative findings are the findings of Goetz from Minnesota, who using insulin with the same technic, did show a widening of the A-V differences. Fajans and others have pointed out that the glucose tolerance does not improve. However, if one waits a long enough time it does show improvement.

I would like to suggest that the difficulty in comparing insulin with the sulfonamides is that the amount of insulin ejected by these drugs is small. When one compares that to a single or even continuous injection of the amounts of insulin we usually use, even the small ones, it is a widely divergent comparison. The drugs may stimulate the beta cells to produce very small, continuously secreted amounts of insulin, rather than cause an ejection of the insulin similar to single intravenous injection.

If the drugs act acutely on the liver, then one cannot see why in the depancreatized human or animal there is no action in the presence of liver unless extremely high doses are given for long periods.

We have some data which convince us that one can get a very nice drop in the blood sugar level in an animal from whom the liver has been removed but in whom the pancreatic circulation was left intact, emptying into the vena cava. In the complete absence of the liver, one can get hypoglycemia using these drugs *if the pancreas is present.*

GEORGE E. ANDERSON, M.D., (*Brooklyn*): I should like to say that what Dr. Berson found in rabbits we have found in dogs. In others words, fourteen days after total pancreatectomy, ultimately proved by autopsy on the dog, we were able to find after a single dose of 150 mg. per kg. of Orinase by vein that the animal's arteriovenous difference was practically the same as it had been before the administration of the Orinase. Then the administration of a small dose of glucagon caused a de-

cided rise in the arterial blood sugar, but the venous glucose curve followed rather precisely the arterial without any material change in the arteriovenous difference. At that point, the administration of a minute dose of insulin caused a decided drop in the venous sugar, with the glucagon-produced arterial glycemia still going up. The venous fall persisted, suggesting the initiation of a peripheral insulin effect only after administration of insulin. One must conclude that under the conditions of this experiment, there is no increased transport of glucose across peripheral barriers either directly or indirectly by virtue of the Orinase. The moment insulin was put into the system, glucose transport occurred. We too believe that the action of Orinase is in the main, if not entirely, central—in the nature of cutting down glycogenolysis in the liver.

JOSEPH L. IZZO, M.D., (*Rochester, New York*): If I may be permitted, I should like to hurl a few bricks of my own at Dr. Levine's straw man number four. Dr. Levine has said there is no evidence in the depancreatectomized animal given exogenous insulin that sulfonylureas have any effect. With this I cannot agree. Dr. Haist in Toronto, Dr. Mary Root at the Lilly Research Laboratories and Dr. Goldner in New York City have all observed a reduction in exogenous insulin requirements in depancreatectomized dogs under the influence of sulfonylureas. Also, there was a depancreatized human, a patient of Dr. Chute in Toronto, in whom there was a fall in insulin requirements on giving BZ-55.

Dr. Levine states that the amount of extra insulin released by the pancreas under the influence of BZ-55 may be so small that the methods that have been used are not sufficiently sensitive to detect this. However, Dr. Lukens has found that there is no enhancement of the hypoglycemic response to sulfonylureas in the hypophysectomized cat compared to the normal. This is of interest, since the hypophysectomized cat is very sensitive to insulin and therefore might be expected to show up small changes.

Then there are the experiments of Miller and Dulin at the Upjohn Laboratories. They compared the effects of sulfonylureas and insulin on muscle and liver glycogen. They found that the sulfonylureas increased liver glycogen but not muscle glycogen, whereas insulin increased muscle glycogen but did not increase liver glycogen.

Finally, Dr. Fajans et al. at Ann Arbor compared the effect of sulfonylureas and insulin on blood sugar and blood pyruvate. The hypoglycemia produced by insulin was associated with a rise in blood pyruvate. On the other hand, comparable hypoglycemia produced by Orinase was associated with an initial fall in pyruvate.

The foregoing data would appear to be inconsistent with the hypothesis that the sulfonylureas act principally by stimulating insulin production or insulin release by the pancreas or by suppressing insulinase activity in the liver. They are consistent, however, with other experimental data suggesting that these compounds act, at least in part, by reducing hepatic glucose output.

FREDERICK M. ALLEN, M.D., (*New York City*): It seems clear that any reported increase of insulin production by these drugs is practically unimportant, and diminished destruction of insulin is made improbable by the absence of effect in the very cases in which it should be most pronounced, namely the severe ones which receive fifty to a hundred units of insulin daily. I am impressed by the evidence in favor of an increase of liver glycogen and diminished supply of glucose from the liver, without augmented uptake by the tissues. This means a mechanism opposite to that of insulin, which makes hypoglycemia by stimulation of glucose uptake by the peripheral tissues in spite of increased breakdown of glycogen and discharge of glucose by the liver. At least, a radical difference between the two processes is evident on the most cursory observation of symptoms in animals. Insulin produces in mammals a characteristic series of hypoglycemic convulsions, each controlled by a small glucose injection, and any increase of dosage acts according to a fixed law; namely, the effect is prolonged rather than intensified. On the contrary, the sulfonylurea drugs produce a flaccid depression or coma, and if given along with insulin they intensify the hypoglycemia with clear evidence of an added foreign process. As I stated in greater detail at the Chicago meeting of the American Diabetes Association, it should have been clear from the outset that this drug hypoglycemia is in no sense an insulin hypoglycemia.

BENJAMIN JABLONS, M.D., (*New York City*): Recently we encountered in a patient a response to Orinase which is pertinent to this discussion. This patient suffering from rheumatoid arthritis had been on cortisone therapy for several months. The administration of Orinase 3 gm. for two days produced a drop from her normal blood sugar level to 18 mg. per cent suggesting that adrenal insufficiency from prolonged cortisone therapy may be a factor to be considered in some cases.

ARNOLD LAZAROW, M.D., PH.D., (*Minneapolis*): In regard to evaluating the effects of various physiological factors and chemical agents on the alpha cells, I would like to re-emphasize that we do not yet have a specific histochemical method for the demonstration of glucagon. Until we can be sure that the secretion gran-

ules which are seen in the alpha cell represent stored glucagon, attempts at correlating alpha cell cytology and glucagon content are likely to be fraught with difficulty.

It should also be pointed out that in most of the work that has been carried out thus far, the effect of the sulfonylureas on insulin release has been studied in acute experiments. An immediate effect on the pancreatic insulin output was demonstrated following sulfonylurea injection. I would like to ask if there is any evidence to suggest that in animals receiving chronic sulfonylurea treatment, where the sulfonylurea level has been maintained for several days, whether the pancreas is putting out increased amounts of insulin into the circulation?

EDWARD TOLSTOI, M.D., (*New York City*): Has the factor of activity of the sulfonylureas been at all related to the circulation of the drug?

CHAIRMAN GRAEF: Dr. Goldner, you were the first speaker this afternoon and you cut yourself short. Do you want to add anything?

DR. GOLDNER: No.

CHAIRMAN GRAEF: Dr. Levine, do you want to add anything?

DR. LEVINE: Just one thing. I want to answer Dr. Tolstoi. Yes, if it is circulated, it is inactivated. The circulation inactivates, and the degree of circulation goes with inactivity. Let's all hope by a year from now we will have resolved these problems.

CHAIRMAN GRAEF: Dr. Cox, do you care to add anything? Dr. Berson, you presented a divergent view, and you have been subject to review. Would you like to comment in rebuttal?

DR. BERSON: It is not a comfortable position for a discussant to be a defendant. However, it is gratifying to see that Dr. Levine is somewhat in the same position as we are. We found it difficult to explain our data if the presence of some of the beta cells is required for the action of sulfonylureas, and I think Dr. Levine finds it difficult to explain away our data. However, I am not sure that the beta cells have to be present to show some demonstrable effect with the sulfonylureas.

There is a recent report in the *Canadian Medical Association Journal* (Sirek et al. 74:960, 1956) in which a totally pancreatectomized dog could be maintained with BZ-55 at normal fasting blood sugar levels for as long as ten days while receiving no insulin, although in the previous experience of this group, preliminary signs of coma always appeared within seventy-two hours in depancreatized dogs deprived of exogenous insulin. Therefore, I am not sure that we have to accept the conclusion that the sulfonylureas cannot reduce blood sugar whatsoever if the beta cells are absent.

The Vitamin B Complex in Carbohydrate Nutrition

It is now known that the vitamin B complex plays an integral part in carbohydrate metabolism and that the need for this group of vitamins depends upon the amount of carbohydrate eaten. Why was knowledge of its existence not acquired much earlier in human experience, and why did the race not suffer from that lack of knowledge? The answer to these questions is that it was only in comparatively recent times that the natural union between the vitamin B complex and carbohydrate, a union existing in whole grain and plants, was broken by the industrial processing of foods. Before this occurred, the supply of the B vitamins was automatically adjusted to the amount of carbohydrate eaten; the occurrence of vitamin B deficiency with its consequent disturbance in nutrition is, therefore, a comparatively recent development in the Western World. In the Orient, the earlier large-scale introduction of polished rice led to the first known instances of vitamin B deficiency (beriberi) and, indeed, to the first recognition of the existence of this group of vitamins.[1]

From the book *Modern Nutrition in Health and Disease* edited by Michael G. Wohl, M.D., and Robert S. Goodhart, M.D. Philadelphia, Lea & Febiger, 1955. Chapter "The Role of Carbohydrates in the Diet" by Samuel Soskin, M.D., and R. Levine, M.D., p. 157.

[1] Vedder: Beriberi. Baltimore, William Wood & Co., 1913.

Clinical Experience and Experimental Studies with Tolbutamide

James W. Craig, M.D., and Max Miller, M.D., Cleveland

This conference affords evidence of the intense interest and the thoughtful investigations which have been stimulated by the recent introduction of two sulfonylurea compounds, carbutamide and tolbutamide. A careful study of the effects of these compounds on patients with diabetes mellitus of varying types and etiologies should aid in the assessment of their clinical usefulness and might yield information about their blood sugar lowering mechanism. Such a study will be described in this paper. Detailed descriptions of some of our results have been published previously[1, 2] and will be only summarized.

CLINICAL STUDIES

All of the patients were hospitalized and maintained on a constant dietary intake during the period of study. When insulin was employed it was given as regular insulin twice daily, except in the case of the patient with lipoatrophic diabetes who received 500 units of insulin each day in a single dose. Tolbutamide* was the sulfonylurea compound used for these studies.

The first three patients had diabetes mellitus of undetermined etiology but varied in their clinical characteristics. Each one represented a different type of R. D. Lawrence's three types of idiopathic diabetes mellitus.[3] As previously reported, in a patient with stable or lipoplethoric diabetes, who had required 45 units of NPH insulin daily, the fasting blood glucose concentration and twenty-four-hour glucose excretion were well controlled without insulin by 2 gm. of tolbutamide daily. It is interesting that surgery on this patient was associated with a temporary marked rise in the blood concentration and urinary excretion of glucose in spite of the continued administration of tolbutamide. This patient has now received the drug, 2 gm. daily for ten months, with no apparent decrease in its effectiveness and without toxic manifestations. The results in a patient with labile or insulin deficient diabetes were in sharp contrast to those just described. During the period of study this patient received 70 units of insulin daily instead of her usual dose of 90 units to ensure a persistent hyperglycemia and glycosuria. The administration of 2 gm. of tolbutamide daily in addition to 70 units of insulin produced no significant alteration in the blood glucose concentration or the glucose excretion. The third patient had lipoatrophic diabetes mellitus and required 2,000 units of insulin daily to control her hyperglycemia. Two grams of tolbutamide daily produced a significant lowering of the fasting blood glucose concentration and twenty-four-hour glucose excretion in the absence of exogenous insulin, but adequate clinical control was not obtained with this drug alone. The effect of this dose of tolbutamide on the fasting blood glucose concentration was equivalent to that of 500 units of insulin, but the effect on the glycosuria was much less marked.

The next two patients furnish examples of diabetes mellitus which was secondary to surgical removal of pancreatic tissue. The first of these patients had developed mild, stable diabetes following subtotal pancreatectomy; it was estimated that 80 per cent of the pancreas had been removed. He required 4 to 14 units of regular insulin daily. In the absence of exogenous insulin, 2 or 3 gm. of tolbutamide daily produced a definite decrease in the fasting blood glucose concentration; although the glycosuria was decreased, it was not as well controlled as was the fasting blood glucose concentration. This observation suggested that the drug has its major effect on processes which maintain the concentration of blood glucose in the fasting state, while an influence on the disposal of exogenous glucose is absent or less evident. This effect was also illustrated by a study of his blood glucose changes following the intravenous administration of glucose and fructose. Tolbutamide diminished the blood glucose rise which was associated with fructose administration, but did not alter the rise which was produced by giving glucose. The second of these patients

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*Orinase® was supplied by The Upjohn Company.

was a thirty-six-year-old woman who developed diabetes mellitus following total pancreatectomy. There was no family history of diabetes mellitus and the patient was quite thin. For twenty years she had had symptoms of chronic pancreatitis. On April 18, 1956, a total pancreatectomy was performed; the excised organ was the site of severe chronic fibrous and calcific pancreatitis. Following pancreatectomy, the patient developed diabetes mellitus which was characterized by marked lability with the occurrence of both ketosis and hypoglycemia, and required up to 60 units of insulin daily. A trial on tolbutamide was conducted in June and July, 1956. During the control period, the daily insulin dose was reduced to 25 units to ensure a persistent hyperglycemia and glycosuria; crystalline insulin was given before breakfast and supper. As shown in table 1, the administration of 2 gm. of tolbutamide daily for eleven days in addition to 25 units of insulin did not produce a significant alteration in the fasting blood glucose concentration or the glucose excretion. It is interesting that no potentiation of the effect of exogenous insulin was demonstrated. Such a finding casts some doubt upon the possibility that tolbutamide exerts its effect by an inhibitory action upon an insulin antagonist or destroyer.

Another instance in which diabetes mellitus may have been due to pancreatic damage, presumably with insulin deficiency, was that of a sixty-seven-year-old man with generalized hemochromatosis. Diabetes mellitus had been diagnosed in 1944 and the diagnosis of hemochromatosis had been confirmed by a liver biopsy in 1949. He had recently been maintained on 55 units of NPH insulin daily. The effect of tolbutamide administration in this case was studied at the Veterans Administration Hospital in Cleveland, Ohio, by Drs. Reginald A. Shipley and Paul E. Wisenbaugh. When the insulin dose was reduced to 25 units daily, marked hyperglycemia and glycosuria occurred. The addition of tolbutamide in doses up to 4.5 gm. per day had no demonstrable effect on blood or urine glucose content. A single intravenous dose of 2 gm. of sodium tolbutamide produced no lowering of the blood glucose concentration 4.5 hours after injection.

TABLE 1
Effect of tolbutamide in a totally pancreatectomized patient

Material	Number of days	Glucose	
		Fasting blood mg./100 ml.	Urine gm./day
Insulin	11	433±61(S.D.)	22±12
Insulin+ Tolbutamide	11	435±36*	31±13†

*p>0.5
†p=0.1-0.05

The next two patients had diabetes mellitus from increased amounts of adrenal cortical steroids being present. The first of these patients was a fifty-four-year-old woman with classical Cushing's syndrome, diagnosed first in 1953. In 1954 classical symptoms and signs of diabetes mellitus appeared and insulin therapy was started. She had recently been taking 24 units of insulin daily. As shown in figure 1, tolbutamide reduced the fasting blood glucose concentration and urinary glucose excretion both before and after the removal of an adrenal cortical adenoma. A fifty-two-year-old man with bilateral idiopathic optic neuritis developed hyperglycemia and glycosuria while receiving 300 mg. of prednisolone daily as therapy for the ophthalmologic condition. His family had no history of diabetes mellitus, and he was not obese, but he required as much as 40 units of insulin daily. At a time when the dose of prednisolone was reduced to 150 mg. and he also received 40 units of ACTH gel daily, he was given a single intravenous dose of 2 gm. of sodium tolbutamide. Within three hours his blood glucose fell from 231 mg. per 100 ml. to 137 mg. per 100 ml. The effect of tolbutamide administration over a longer period of time was not studied in this case of steroid diabetes. Thirty days after the last dose of prednisolone and seventeen days after ACTH had been discontinued, the intravenous administration of 2 gm. of tolbutamide produced a fall in the blood glucose concentration from a fasting level of 64 mg. per 100 ml. to 42 mg. per 100

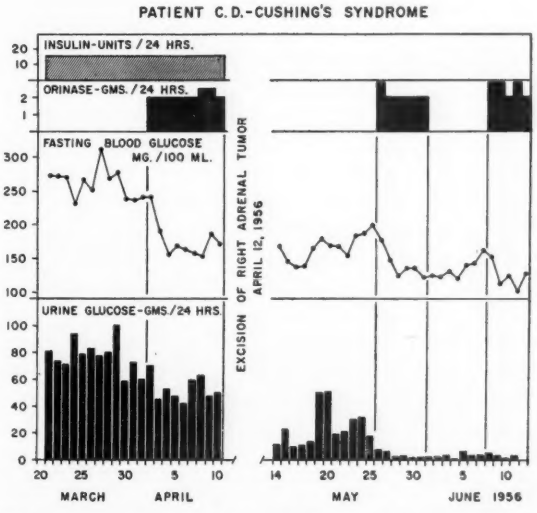

FIG. 1. Effect of tolbutamide on fasting blood glucose concentration and 24-hour glucose excretion in a patient with Cushing's syndrome before and after removal of an adrenal cortical adenoma. During the postoperative period the patient received 25 mg. of cortisone daily.

ml. in thirty minutes; at the end of three hours the blood glucose level was 74 mg. per 100 ml. It appears that the presence of increased amounts of adrenal cortical steroids does not interfere significantly with the hypoglycemic effect of tolbutamide.

SUMMARY AND CONCLUSIONS

1. The blood sugar lowering effect of tolbutamide has been studied in patients with diabetes mellitus of varying types and etiologies. A blood sugar lowering effect of the drug was demonstrated in a partially pancreatectomized patient, but hyperglycemia and glycosuria were not reduced by tolbutamide in a totally pancreatectomized patient who received a constant dose of insulin during the period of study. The drug was effective in decreasing the blood glucose concentration in a patient with Cushing's syndrome, including diabetes mellitus, and in another patient with steroid diabetes secondary to prednisolone administration.

2. Some suggestions regarding the mechanism of action of tolbutamide have been made on the basis of clinical observations. The drug appears to diminish the endogenous formation of glucose without altering the rate of utilization of administered glucose. An inhibitory effect upon an antagonist or destroyer of insulin seems unlikely. The action of the drug is not altered significantly by adrenal cortical steroids.

SUMMARIO IN INTERLINGUA

Experientia Clinic e Studios Experimental Con Tolbutamido

1. Le reduction del nivello de sucro sanguinee effectuate per tolbutamido esseva studiate in patientes con diabete mellite de varie typos e etiologias. Le effectuation de un reducite nivello de sucro sanguinee per le droga mentionate esseva demonstrate in un partialmente pancreatectomisate, sed hyperglycemia e glycosuria non esseva reducite per le administration de tolbutamido a un totalmente pancreatectomisate patiente qui recipeva un dose constante de insulina durante le periodo del studio. Le droga esseva efficace in reducer le concentration de glucosa sanguinee in un patiente con syndrome de Cushing, incluse diabete mellite, e in un altere patiente con diabete steroide secundari al administration de prednisolona.

2. Certe ideas in re le mechanismo del action de tolbutamido es presentate super le base de observationes clinic. Il pare que le droga reduce le formation endogene de glucosa sin afficer le rapiditate del utilisation de glucosa de administration exogene. Il non es probabile que il se tracta de un effecto inhibitori super un antagonista o destructor de insulina. Le action del droga non es alterate significativemente per steroides adrenocortical.

ACKNOWLEDGMENT

The authors wish to thank Drs. Charles Christian and Lawrence Hutchison for their valuable assistance in the study of some of the patients described in this paper.

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DISCUSSION

WILLIAM R. KIRTLEY, M.D., (*Indianapolis*): Much of the material that Dr. Miller has presented follows very closely our own experience. Some of the data that you saw toward the end of his presentation has been analyzed, and I hope to give you a little more specific data of that sort. I might preface my remarks by saying that all of the data presented here have to do with carbutamide.

The observation of our patients now extends over a year's time. We have a few people who have been on the drug for a year, a larger group for eight months. All of those in this series have been on the drug for at least three months. The cases reported here were the ones treated successfully with BZ-55. By this, I mean there was an adequate therapeutic response and no evidence of toxicity.

The cases are broken down into age groups, and, of course, in the majority of instances the successfully treated cases are in the older age group.

The dose of the drug in this group of patients has been, for the most part, between 0.5 and 1.5 gm. per day. In a few instances we have gone a little higher, primarily as a result of our earlier experience, in an attempt to determine whether or not increased dosage was more efficient; and we found that in the majority of cases there was no improvement in therapy by doubling or tripling the dose. In three instances we have gone to 3 gm. per day and these patients have been carried on this dose successfully for a period of weeks.

It is interesting to see that there is no real criterion as to the amount of insulin that might be replaced. The majority of patients who did best fell in the group

requiring less than 40 units per day, but three patients were requiring over 100 units per day. They have been taken off their insulin completely and are on BZ-55 alone and are doing equally well.

We have had failures, of course. The distribution of failures for the most part falls in the younger age group which require the larger insulin doses. But, strangely enough, one individual seventy-eight years of age who had had diabetes for only one year and required 30 units of insulin did not respond at all. You cannot judge every case simply by knowing the history and the type of diabetes that exists.

Because of the fact that this particular drug is a sulfonamide derivative, we were extremely interested in the possibility of toxicity. We heard of a death occurring in a patient receiving carbutamide. This was the first example, and it did not occur until after we had been working with the material for over six months. At that time we decided to investigate the occurrence of side reactions to the drug, and requested all investigators to supply us with data on their patients—not on the deaths, of course, but all types of side reactions. In our own experience we had had two cases of skin reaction and one case of leukopenia.

Reports were received from 1,319 physicians. The total number of cases reported at this time was 7,193. There were 389 side reactions reported, with a reaction per cent of 5.36.

The types of reactions for the most part followed those seen with other sulfonamide drugs. The majority were reported as being skin rashes of various sorts, many of them relatively mild, which could be controlled by the use of antihistamine drugs. Anorexia, nausea and vomiting were rather common complaints. They were not considered as being too serious, and usually cleared up as soon as the drug was stopped. But other things were of more importance, particularly agranulocytosis and leukopenia. Obviously, we were concerned about the possibility of changes in the white blood cell count.

In comparing the incidence of reaction with other sulfonamide drugs, it may be stated in general that they have been shown to have an over-all reaction rate of about 5 per cent, which fits in very nicely with the percentage that we have seen in our own group.

I might digress slightly and enlarge a little on the statements from Germany. The reported incidence of allergy in Germany is 1.8 per cent. Whether there is a difference between the allergy or the reaction in this country and that in Germany, I think no one knows certainly. In fact, the German clinicians are at a loss to explain it. Professor Achelis suggests that perhaps Americans take more medicine and more sensitivity is set up than occurs in Germany. This might account for it.

This is the most important point that I want to make at this time. Fatalities have been reported to occur in patients while receiving carbutamide. I want to point out at the very beginning that these were the reports as received by the clinician handling the case. In most instances we have not had an opportunity to follow up completely to determine the cause of death. The best that we can say at the present time is that it would appear that the drug contributed to the demise, because there are other relevant circumstances in almost every case. But it brings us to the point which Dr. Goldner made earlier, first, that it does no harm. If this were the only means of treating diabetes, of course there would be no question about it, it would be the drug of choice. But since we have other technics and other methods for treating diabetes, we do not know its exact place in the therapeutic methods we have at hand; how far should we go with it?

It is extremely interesting to me, at least, that in less than a year's time there has been tremendous progress in understanding the effects of these drugs, especially since less than a year ago we were quite skeptical about there being any blood sugar lowering effects at all. I can only make a plea for patience to see what happens, with the hope that the mode of action, if finally determined, will give us a new clue to therapy in diabetes.

Perhaps the unfortunate occurrences can be bypassed by other modifications and other means, and we can finally come up with a tool which is going to be effective in a large share of the diabetic population.

HENRY DOLGER, M.D., (*New York City*): My clinical experience with Orinase covers some 500 patients and more. Four hundred patients have been observed in my practice, and 100 cases in the Mt. Sinai Hospital Clinic. Four hundred have been treated for six months, and 100 for nine months.

At first a test response to Orinase was tried and as you may recall from a paper of Drs. Mirsky, Diengott and myself, an interesting graph was created which Dr. Best and Dr. Wrenshall then adapted to their presentation of the available insulin concentration in the pancreas. May I have that one slide, please? This slide represents the response to Orinase over a five-hour period, given in 50 mg. doses per kilo in 200 patients of scattered age and duration of diabetes, from children to older adults. The length of the line up and down

is the degree of response. The longer the line, the better the response to Orinase, and the better the drop in blood sugar.

The little circles indicate failures, and you see the failures are limited primarily to those where the age of onset is below twenty, and where the duration of diabetes is also of increasing magnitude. In other words, the lines are best in the back and best in the far left.

This slide is identical with Dr. Best's and Dr. Wrenshall's. I stopped using this test as an index of therapeutic response, and have abandoned this technic of testing patients beforehand, in patients taking insulin, because the only thing that counts is the therapeutic effect itself as insulin is reduced and then discontinued with Orinase treatment.

The patients are chosen at random without regard to preconceived ideas about obesity, duration of diabetes or insulin dose. The only thing which means anything to me is the age at the onset of diabetes. I am reluctant to treat anybody whose diabetes began below age twenty, although I have tried this out in some thirty-odd patients. Occasional good responses in juvenile cases who are able to stop insulin for, say, six months are rare.

By and large the results were as follows: In the group with age of onset of diabetes below twenty, I have had no dramatic result with Orinase, regardless of dose or manner of administration. In the age group twenty to forty, 30 per cent have completely abandoned insulin, with very good control. The patients seem to be better controlled with Orinase than before we had this material. Ninety-five per cent of the diabetic population is in the group with age of onset over thirty or forty, and 75 per cent of these patients can be helped.

Another thing about the clinical management is that the range of dosage is much narrower than with insulin. The average therapeutic dose of Orinase runs between 1 and 2 gm., and occasionally as high as 3 gm. I have several patients on 6, 10 and 12 gm. One patient has taken 12 gm. for six months without any sign of toxicity. In the group on 1 or 2 gm. a day, the incidence of toxic reactions has been less than 1 per cent. There was no significant leukopenia. The only side reactions I have seen were three skin rashes. One turned out to be scabies, one urticaria, and one erythema. The most disturbing of all reactions occurred in three patients who complained of marked facial redness after alcoholic liquors had been taken along with Orinase. This would be an asset for Dr. Joslin's anti-alcohol crusade, I am sure.

The entire series, to date, seems very promising and I feel that at least with respect to Orinase, there is no hazard in its administration. The effective therapeutic results require a great deal of concerted care in following the patients. Those who are on insulin have to have their dose reduced gradually. I hate to see anybody requiring Orinase put into the hospital for initial therapy. Patients who are not taking insulin can be managed much more easily. The primary aim is to find the minimum effective dose which results in reduction of hyperglycemia and abolition of glycosuria.

In patients with recently discovered diabetes, it is remarkable that Orinase can do as well in the initial treatment as insulin. In other words, these patients in the age group of thirty, forty and fifty years, with a daily dose of 3 gm. of Orinase, can gain 12 lb. in three weeks and will have complete abolition of all symptoms and remarkable restoration of well-being. Whether this satisfies Dr. Levine or Dr. Berson is immaterial. For us as clinicians, it works like a charm, I mean, it works like insulin in these patients. Whenever this material will be available for general use, the only hazards I could possibly envisage would be the matter of overdosage and the indiscriminate use of these tablets by patients themselves. A patient, in the mistaken belief that Orinase was a weight-reducing substance, took thirty-six tablets in one day. She lay in bed for three days with mild hypoglycemia. We have since discouraged these people from confusing Orinase with Slenderella!

THOMAS H. MCGAVACK, M.D., (*New York City*): From a clinical viewpoint, the advent of the sulfonylureas has placed emphasis upon the necessity for and importance of some method of classifying patients who have diabetes mellitus. I think Dr. Miller's paper is an excellent addition to this objective, separating, to some extent, those who will and those who will not respond to these drugs in accordance with the clinical classification of Lawrence.

Our largest experience has been with Lawrence's lipoplethoric group. About two-thirds of the patients we have studied fall in this group, but before discussing them, I shall mention one instance of striking lipoatrophy in a woman, whose diabetes began at the age of thirty-four, and lipoatrophy shortly thereafter. Her severe bouts of anorexia, the hyperlipemia, the marked ketosis and occasional acidosis, the episodes of extreme resistance to insulin and the hepatomegaly completed the classical picture as described by Lawrence.

Quite unlike Dr. Miller's case, this individual not only did not respond to tolbutamide but on being given tolbutamide, without any change in the dose of insulin, became more anorexic, and developed a severe keto-acidosis, which required very vigorous treatment.

We have analyzed data from forty-one patients, about one-third of whom belong to Lawrence's insulin sensitive group and the remainder to the lipoplethoric group. I only wish to emphasize one point in relation to our clinical management of these two groups.

We were unable to tell a priori which ones would respond to the administration of a sulfonylurea compound and which ones would not. What experience have you had, Dr. Miller, with any of the "tolerance" tests available for predetermining the suitability of cases for use of these drugs?

Let us pass from the over-all response of these forty-one individuals to a study of their thyroid function. We became concerned about thyroid function because one of these drugs is a sulfonamide and the other is closely related chemically. When doses of 1 gm. daily of either drug were employed for periods of time up to twenty-one weeks, no signs of change in thyroid function as determined by radioactive iodine uptake were observed. When we advanced the dose of tolbutamide to 2 gm. daily, we found that for periods of time up to twenty-one weeks, there was no significant change (S.D. $\pm$ 4.3), the uptake being reduced on the average from 22 per cent to 19.5 per cent.

However, when 2 gm. of carbutamide were used daily, the average uptake of I^{131} dropped from 21.6 per cent in the control period to 8.7 per cent at the head of nine weeks of therapy and remained low in some patients observed for as many as twenty weeks while function in others had returned to normal. It is clear that carbutamide definitely lowers thyroid function. Where the initial level of dosage lies for such an effect to become clinically detectable is not settled. Both Dr. Kirtley and Dr. Irwin have had experience with 1.5 gm. doses of carbutamide and found no change in radioactive iodine uptake after thirty weeks of treatment.

In conclusion, I believe our use of these hypoglycemia producing drugs should be guarded until three matters have been further clarified: (1) the incidence and nature of severe toxicity; (2) the influence on thyroid function for longer periods of administration, and (3) satisfactory methods for the selection of suitable cases.

BRUNO W. VOLK, M.D., (*New York City*): As a pathologist I have to ask the question whether the patients who died have been autopsied. There are quite definitive morphologic characteristics in patients who die as a result of sulfa administration.

I do not think we should be satisfied with a table which mentions only the number of fatalities. I think it is important to point out the cause of death as established by post-mortem examination. Did the patients die while they were under the sulfa therapy?

MARTIN G. GOLDNER, M.D., (*New York City*): A few words might be in place about the question of dose-equivalents between insulin and the hypoglycemic sulfonylureas.

Dr. Kirtley mentioned one instance where 500 units of insulin could be replaced by 1 gm. of carbutamide. This was a case of insulin resistance which required 1,000 units of insulin per day for control and even when carbutamide was used, the insulin dose could only be cut in half, not replaced entirely. One certainly would gain a wrong impression about the potency of the hypoglycemic sulfonylureas, if a general conclusion were to be drawn from this observation. It seems to be rather an exception than the rule that the sulfonylureas are effective at all in insulin resistance. In general, it appears that not more than 20 to 30 units of long-lasting insulin can be replaced by the oral drugs. Thus, only those patients whose daily insulin requirement is not higher than 20 or 30 units are likely to be transferred to the sulfonylureas and to be taken off insulin. Where the requirement is higher, the use of the sulfonylureas may permit decrease of the dose, but will rarely free the patient from the injection of a basic amount of insulin. This, of course, applies only to that type of diabetes which is responsive to the sulfonylureas at all; in the juvenile diabetes and in surgical diabetes not even a sparing effect can be expected. It also seems that carbutamide and tolbutamide are not effective to the same degree. In our series at the Jewish Chronic Disease Hospital in Brooklyn, both drugs were used alternatively, and it appeared that 2 gm. of tolbutamide were usually needed to produce the effect of 1 gm. of carbutamide. Carbutamide, as you know, is the more toxic drug, or at least the one with which more often side effects have been observed. Dr. Kirtley has presented the results of his careful survey of these toxic side reactions. As yet, no similar survey has been carried out for tolbutamide, and we will have to wait until we can be certain as it appears now that this compound is harmless over longer periods of time and in the larger doses which seem to be necessary. As far as I know, a few deaths have occurred in Germany, both after carbutamide and tolbutamide. The reports mention only the histological appearance in the islet cells and fail to give the general findings at autopsy. Dr. Volk has stressed the importance of the post-mortem examination. In only two or three of the carbutamide deaths in this country, the autopsies have shown anatomical changes characteristic of sulfonamide damage. Other deaths may have been incidental; after all, the patients in whom

the sulfonylureas have been used most and with best results were elderly people with many signs and symptoms of aging and degenerative diseases.

In a comparison of the efficacy of insulin and of the hypoglycemic sulfonylureas, another factor of clinical importance must be mentioned which is not a true side effect but a serious shortcoming of the new drugs. They are not effective in ketosis or acidosis. If any intercurrent disease develops which is likely to precipitate ketosis or acidosis, the sulfonylureas even in increased dose will be of no help and insulin must be employed immediately. We had occasion to see a patient who had been well controlled on the oral drugs for several months. Then she developed an infection and fever and was admitted to the hospital with severe acidosis.

Stimulating as the work with the new drugs may be, we shall have to learn much more about their mechanism of action before we can consider them as substitutes for insulin. Today it seems rather unlikely that they will be able to replace insulin to any great extent in the management of diabetes and its complications.

MAX MILLER, M.D., (*Cleveland*): I only presented a typical case in each group. I agree with Dr. Dolger, and others, that the management of each case has to be individualized. If there is any single generalization that can be made regarding response, it is the following: Those patients who tend to show ketosis and acidosis on withdrawal of insulin, in other words, who are insulin dependent, do not respond to the drug. I think the case of ketosis that Dr. Goldner described was not due to the drug per se, but to the fact that it would have occurred after the omission of insulin alone. This hazard cannot be overemphasized. We have already seen disastrous results where patients have been transferred to these drugs without a careful history being taken with particular reference to previous bouts of ketosis or acidosis. When patients are first given the sulfonylurea drugs, they should be under careful observation when insulin is being reduced or eliminated, to see whether ketosis develops.

Our studies on the metabolism of C^{14} labeled glucose in stable and labile subjects with diabetes revealed differences in oxidation that could be correlated with the effectiveness of the oral hypoglycemic agents. Two of the patients I described here had been given C^{14} glucose on previous occasions. The patient who responded to the drug oxidized glucose to CO_2 at the same rate as a normal individual. The patient who went into ketosis on withdrawal of insulin and did not show any reduction of blood sugar after tolbutamide administration oxidized C^{14} glucose to CO_2 at half the normal rate.

We have suggested that the metabolic defect in the stable diabetic group is overproduction of glucose, presumably by the liver. On the other hand, the defect in labile diabetes seems to be underutilization, which perhaps can be correlated with insulin deficiency. It is tempting to conclude that the sulfonylurea drugs act primarily on the liver to cut down the overproduction of glucose.

It is interesting that Dr. Stetten has indicated he thought that the hyperglycemic state produced by cortisone was the result of gluconeogenesis, or overproduction of glucose in the liver. The steroid diabetes cases that I described also responded to the drug. In other words, the clinical information seems to fall in line, in this respect also, with the idea that tolbutamide interferes with glucose production in the liver.

The major objection to this theory is the consistent finding in *human* depancreatized patients that no detectable effect on blood sugar can be demonstrated. Our own case of a totally depancreatized patient reported today showed absolutely no response. Evidently the presence of the normal secretions of the pancreas seems to be a sine qua non for a measurable action of the sulfonylurea drugs. Continued research is essential if these apparently disparate observations are to be reconciled in one over-all theory.

No discussion of the possible clinical role of these new oral hypoglycemic agents would be complete without a critical evaluation of their toxicity. In the final analysis this will be the most important single factor that will determine its place in the everyday management of diabetes mellitus. Dr. Kirtley has already described the toxic manifestations associated with the administration of carbutamide. For purposes of comparison, the untoward reactions occurring with tolbutamide in a similar number of patients are listed in table 1 (data on tolbutamide supplied by Dr. C. J. O'Donovan as of May 1, 1957).

The blood changes so far reported with tolbutamide

TABLE 1

Comparison of Toxic Manifestations of Carbutamide and Tolbutamide

	Carbutamide*	Tolbutamide
Number of U. S. cases reported	7,193	7,147
Blood changes	67	13
Skin changes	109	67
(Exfoliative dermatitis)	6	0
Drug fever	79	0
Fatalities	8	1

*Data of Dr. Kirtley.

include only leukopenia in thirteen cases, but without agranulocytosis. In no instance did clinical symptoms or signs appear. There were no cases of anemia or thrombocytopenia. The skin changes were of varied nature with tolbutamide, usually of short duration, and often clearing in the face of continuing administration of the drug. Exfoliative dermatitis did not occur. There were no reports of hypothyroidism or of drug fever. The one fatality was associated with hypoglycemic coma in an eighty-four-year-old man who apparently took an unknown amount of tolbutamide during a period of six days when he was not under direct medical supervision. Obviously the same precautions and supervision are as essential with these new oral hypoglycemic agents as with insulin.

These comparisons indicate that tolbutamide so far has not exhibited the same type of serious toxic reactions found with carbutamide. Consequently, it is only fair to predict that the former drug will have some place in the management of the older, stable-type patient whose diabetes is not too severe and who does not develop ketosis when deprived of insulin. Dietary control of the obese individual will remain as essential as before and the fact that the sulfonylurea drugs seem to exert their effect primarily on endogenous glucose formation also emphasizes the need to avoid dietary excesses. Patients taking the drug must be kept under continuous observation in order to detect quickly the possible appearance of any new type of toxic reaction. The physician must also be aware that stress situations, such as trauma or infection, may worsen the diabetic state and ketosis may develop, with resulting ineffectiveness of the oral drugs. Under such circumstances the physician must be on the alert to step in with insulin to avoid disaster.

CHAIRMAN GRAEF: Thank you, Dr. Miller.

We come to our last presentation. I think I probably speak for most of you here when I say that we need a long view from the bridge. Are we on a bridge to a new form of therapy of diabetes, to an entirely new approach to the selection of patients for control? Can we now envisage a degree of control or prevention of the vascular complications?

Incidentally, you all, I am sure, noted that no reference was made, presumably because of the lack of long-range observations, on the effectiveness of the oral compounds in inducing cessation or recession of nephropathy, retinopathy or neuropathy.

I don't think I have to say any more about why we need Dr. Best, and it is a pleasure to welcome him here again to give us a "long view" perspective on this subject.

Closing Discussion

CHARLES H. BEST, M.D., D.Sc., (*Toronto, Canada*): I regret that another meeting in New York this morning prevented my attending the earlier session. I was particularly sorry to miss the presentation of Dr. Lazarow. My imagination has always been stimulated and my understanding increased by his contributions to our knowledge of the pancreas and of insulin. I suppose, however, that all recent evidence has confirmed and strengthened our opinions, formed many years ago, that insulin is a respectable physiological hormonal agent. It possesses all the qualifications of a hormone, which I need not discuss in detail here.

Certainly the work on glucagon has not yet reached the stage when the same can be said about it although great strides have been made. I have read the excellent paper that Dr. Bromer presented. This is a triumph indeed. We now know in detail the structure of glucagon.

I am sorry that I did not hear my friend, Dr. Anderson, discuss the interrelationship of insulin and glucagon, but I have to insist that we are on much stronger grounds when we study variations in insulin effect, using all available procedures, than we are when we investigate what may be the effects of glucagon. It has been inferred that the variations in blood sugar which are caused under certain circumstances when growth hormone is given, are due to glucagon liberation. There is little evidence for this as I will discuss in a moment.

Dr. Salter presented our recent work on the effects of an excess of glucagon, and he feels very strongly, as I do, that these are good observations but not physiological ones. Both Dr. Salter and I agree with Dr. Herbert Evans, that the evidence does not indicate that the effects of these large doses of glucagon represent a physiological action, but the findings are extremely stimulating and may be of some physiological interest.

We wish to press ahead, to give smaller doses, and

to see if any aspect of these new findings can be co-ordinated with physiological processes.

The effects of injection of growth hormone on blood sugar in dogs are extremely interesting. You will perhaps remember the paper by Bornstein, Reid and Young and the communications by Dr. Foa which demonstrated that a substance producing hyperglycemia was liberated into the pancreatic duodenal vein of rats and dogs after administration of growth hormone.

Some time ago Dr. Sirek and I showed in dogs that the abrupt rise obtained in blood sugar after growth hormone could be prevented by dihydroergotamine. The action of glucagon is not prevented by this drug. Therefore, this effect was probably due, in small part, if at all, to glucagon.

More recently Dr. and Mrs. Sirek have shown that one can dispense with the pancreas completely. If you take blood from the preserved pancreaticoduodenal vein you get exactly the same effects as if the pancreas were there. The hyperglycemic material therefore does not come exclusively, if at all, from the pancreas.

Recently, Dr. Anna Sirek has experimented with 5-hydroxytryptamine (Serotonin). She has shown, for the first time, that this substance causes a very pretty rise in blood sugar in depancreatized dogs. Perhaps this is the substance liberated, and not glucagon at all. Her data show some respectable rises in blood sugar when you give 5-hydroxytryptamine to depancreatized dogs. There are many points to be worked out and there is yet no proof whatever that 5-hydroxytryptamine is liberated by growth hormone. We hope to explore this possibility.

I find myself quite undecided about the physiological role of glucagon. The chemistry has been advanced and there are many, many opportunities to add to our knowledge of the physiological action. When we attempt this, we must be certain that we *are* studying the effects of glucagon.

Now we come to the sulfonylureas, and I must not recapitulate statements which have been made by so many authorities this afternoon. I have listened with great pleasure to Dr. Goldner, Dr. Cox, Dr. Miller and Dr. Levine, and those who discussed their papers. There are many indications from these and other communications that the production of sugar in the liver is inhibited by these sulfonylureas. The activity of a rapidly increasing list of enzymes which function in liver tissue has been shown to be decreased by administration of the sulfonylureas.

Dr. Berson reviewed this particular field extremely well. There is no doubt that in many species, as Dr. Levine emphasized, there is a stimulation of insulin liberation. I think, as Dr. Dolger pointed out, that there probably is a stimulation of insulin liberation in some human patients — presumably the group in which Dr. Gerald Wrenshall has demonstrated residual pancreatic insulin. This may be very small, however, and under some circumstances, I think the evidence indicates that it is very small indeed. I am thinking only, of course, of the cases in which the drugs produce an effect.

In answer to Dr. Lazarow's question, Dr. Ashworth, working with Dr. Haist, has given BZ-55 over a long period of time and has seen a definite increase in the islet volume of the rat pancreas.

It may be that the safe therapeutic action of the sulfonylureas has to do with the liberation of insulin and perhaps with the stimulation of the insulin-producing apparatus. In depancreatized dogs, there is a very slim margin between the amount of sulfonylurea which exerts an antidiabetic effect and the amount which is very definitely toxic. A dose which exerts no antidiabetic effect may be toxic. I think it was Dr. Izzo who referred to a publication from our laboratory in which it was stated that a depancreatized dog on BZ-55 was receiving no insulin at all. The effect of the sulfonylurea on the blood sugar was very definite. Mrs. Sirek has now studied a number of these dogs. The adult dogs may go on for several months before signs of toxicity appear. Mrs. Sirek has also treated depancreatized puppies. Two of these puppies have died suddenly with degeneration of the liver and widespread signs of hemorrhage. The histological picture of the liver indicated a very definite toxicity. In the two depancreatized puppies and in two adult dogs the prothrombin levels were reduced.

This effect on the clotting mechanisms was also drawn to my attention by Dr. Hallas-Møller and a paper from his laboratory by Dr. Per Schambye is now on press.* Before this, however, as we have reported in the *Journal of the Canadian Medical Association,* a depancreatized dog had been maintained on BZ-55 without insulin. This was the first finding which alerted us to the possibility of liver damage. Somewhat later, actually before I received word of Schambye's findings, Mrs. Sirek had observed signs of generalized bleeding in her depancreatized dogs. There were, of course, other possible explanations but the evidence is now becoming quite definite that liver injury plays a role in the effect of BZ-55 in depancreatized dogs.

Dr. Per Schambye has used somewhat higher doses

* This paper and one from our laboratory covering a similar field have now been published in DIABETES 6, 146 and 151, 1957.

than Mrs. Sirek and he has seen more advanced liver degeneration with the profound bleeding tendency. Liver involvement has been suspected in some human cases but I know of no record of such definite effects as those observed in depancreatized dogs.

I would think, from everything that I have heard and read, that there are probably two actions of these sulfonylureas: (1) the liberation of small amounts of insulin with perhaps a stimulation of insulin production, and (2) a mild or chronic toxic effect on the liver. This may prove to be so mild that it can be ignored therapeutically. But the signs in these depancreatized puppies are reminiscent of what we saw years ago with Synthalin, which was certainly a potent liver poison. The sulfonylureas have a much more subtle action and of course one may be found which is acceptable therapeutically in certain cases. I have to emphasize that our observations are with carbutamide only*—and only in the dogs. The story may well be quite different with other compounds and many of these will certainly be studied. A great deal of what was said today should encourage chemists and clinicians to find a safer and more effective stimulant of insulin production in the cases which still have the mechanisms for this process available in their pancreas.

We may be unduly impressed by findings in depancreatized dogs. Similar toxic effects have not been clearly seen with doses equivalent to those used therapeutically in other species. But the dogs have helped us many times. I am no authority on clinical matters, but I do not think it would be in the best interest of diabetics to recommend widespread general use of any of the sulfonylureas at present available.

There are already a number of experimental studies, as yet unpublished, which suggest that Orinase also may be exerting at least a part of its effect on the liver. The absence of toxic effects of this substance in the patients, who have not, I believe, been observed as long as those on BZ-55, should also be stressed. I predict that the next year will reveal many new facts about the agents now being studied and, I hope, that new and even safer adjuvants will be developed. There is certainly a place for the perfect one.

* Dr. A. Sirek, in confirmation and extension of the findings of Dr. Schambye, has now observed that Orinase, given in amounts comparable to those recommended for clinical use, produces in depancreatized puppies treated with insulin, raised serum alkaline phosphatase and prothrombin times and a lowering of plasma proteins. These effects are similar but less severe and slower in onset than those produced by BZ-55 in depancreatized dogs.

What is Obesity?

The specification of "optimal" or "ideal" weights, as well as diets, is a hazardous business. For body weight and relative obesity, at least, the only point on which there will be full agreement is that major dedepartures from the population average should be avoided. There is no doubt that there is an excess mortality penalty in later life associated with marked overweight at the time of application for life insurance. However, the primary data are for major degrees of overweight, from 20 to 75 per cent above the standard average body weight at given height and age, the majority apparently being something like fifty pounds heavier than the average of the population. But to suggest that the major national health obstacle in the United States is obesity because perhaps a tenth of the population may be 10 per cent or more above the average body weight[1] is more than can be sustained from present evidence. Is there actually any serious health hazard necessarily associated with 10 per cent overweight? From what causes? At what ages? And can we disregard the question of obesity versus overweight? Much more research is needed before scientifically acceptable answers to these questions will be at hand. One thing seems certain. The elimination of gross overweight among Americans cannot, by itself, be expected to bring our adult mortality experience to a level to compare favorably with that in such countries as England and Wales, the Netherlands, Italy and the Scandinavian countries.[2] A more penetrating analysis of obesity, rather than mere body weight, might reveal more scope for improvement.

From the book *Modern Nutrition in Health and Disease* edited by Michael G. Wohl, M.D., and Robert S. Goodhart, M.D. Philadelphia, Lea & Febiger, 1955, Chapter "Body Weight, Body Composition and Calorie Status" by Ancel Keys, Ph.D., pp. 29-30.

[1] Armstrong, Dublin, Wheatley, and Marks: J.A.M.A. 147:1007, 1951.

[2] ————: Am. J. Pub. Health 43:1399, 1953.

EDITORIAL

THE CLINICAL USE OF TOLBUTAMIDE IN DIABETES MELLITUS: A STATEMENT OF THE AMERICAN DIABETES ASSOCIATION*

Tolbutamide (Orinase), a hypoglycemic agent for oral use, recently has been released by the Food and Drug Administration and is being generally distributed for use by prescription. Members of the medical and allied professions should be informed concerning this new drug.

It is a sulfonylurea compound with the empirical formula $C_{12}H_{18}N_2O_3S$. It lowers the blood sugar of normal animals and man and of some, but not all, diabetic patients. The mechanism involved is still unknown, but it seems clear that it is ineffective in the complete absence of insulin. Hence it cannot be considered a true substitute for insulin.

According to the manufacturer, experience with more than 5,000 cases has revealed no deaths clearly attributable to the drug during the 1½ years that it has been employed in this country. Toxic reactions, none of them serious thus far, have occurred in approximately 3 per cent of the cases. They have consisted chiefly of gastrointestinal disturbances, cutaneous eruptions presumably due to hypersensitivity, headache and some intolerance to alcohol.

Tolbutamide is contraindicated in those patients with onset of diabetes in childhood or adolescence, those with unstable diabetes, those with a history of diabetic coma, those undergoing surgical operations, or those with existing complications such as ketosis, acidosis, infection, severe trauma, disease of the liver, thyroid or kidneys, or any other condition that usually increases requirement for insulin. In such situations insulin is essential, and attempts to replace it with tolbutamide would be dangerous. There is little or no published information concerning the effect of this drug in pregnancy.

There is, of course, no point in prescribing the drug when diabetes can be controlled with diet alone.

Tolbutamide is most effective in adult patients with relatively mild diabetes who have required small to moderate doses of insulin. The best test for responsiveness is the administration of the drug for a period of seven days during which insulin is withdrawn gradually and tests of the urine for glucose and ketone bodies are performed three times daily.

The dosage of tolbutamide and the method of attempting its substitution for insulin vary with circumstances. Ordinarily, 3 gm. of the drug are given on the first day, 2 gm. on the second and 1 gm. on the third. This method of initiating treatment is applicable whether the patient has been using insulin or not. Maintenance is provided by divided doses totaling from 0.5 to 1.5 gm. (never more than 2 gm.) daily and must be determined on the basis of experience in each case.

Insulin should never be withdrawn abruptly. In cases in which the previous daily requirement has been less than about 30 units, initiation of treatment with tolbutamide may be accompanied by a simultaneous reduction of 30 to 50 per cent in the dose of insulin, further reductions being made gradually so long as levels of blood and urinary glucose remain satisfactory. Patients who have required more than about 30 units daily may reduce their dose by 20 per cent on the first day of tolbutamide therapy, further reductions being made very cautiously. In daily observation the development, at any stage, of sustained hyperglycemia or glycosuria or any sign of ketosis calls for the abandonment of oral therapy and prompt reversion to maintenance doses of insulin. If the blood sugar remains within reasonable limits, however, oral treatment may be continued, the patient returning for examination at weekly intervals for the first month, then at two-weekly and finally at monthly intervals. The periodic examination should include a urinalysis for glucose and ketone bodies, determination of the blood sugar and a white blood cell count, with a differential count if the latter is low. Determinations of serum alkaline phosphatase and bromsulphalein excretion seem to be the most sensitive tests for suspected hepatic damage.

If not hospitalized, the patient must test the urine at home, informing the physician of any increase in glycosuria. He must be made to understand that close adherence to diet is just as important as when insulin is used.

The patient should be warned about the possibility of hypoglycemia while both insulin and tolbutamide are being taken during the period of stabilization. Combined therapy with both agents for purposes of maintenance is pointless.

*Scheduled to be published in the July 6, 1957 issue of the *Journal of the American Medical Association.*

If side reactions, including gastrointestinal symptoms or allergic manifestations, occur the drug should be discontinued in favor of insulin.

Uncertainty as to the mode of action of tolbutamide and the brevity of experience with it when compared with the many years over which diabetes must be treated require that it be prescribed by physicians, dispensed by pharmacists and used by patients with caution. The manufacturer has prepared an excellent leaflet in which safeguards are given appropriate emphasis. The drug has been released by the Food and Drug Administration for sale on prescription only. This means that, in order to avoid violation of the law, pharmacists who are accustomed to dispensing insulin without prescription to patients familiar with its use must resist any temptation to do so with tolbutamide.

During the past few months a guanidine derivative, temporarily designated DBI, is being tested in clinical and experimental diabetes. This substance belongs to a different chemical family from that of Orinase. The available information is too scant as yet to allow even preliminary conclusions.

It is hoped that the investigators working in this field will be given undisturbed and unpressured time to investigate fully any of these drugs so that premature introduction into general use does not occur.

INFORMATIONAL COMMITTEE ON ORAL HYPOGLYCEMIC COMPOUNDS
AMERICAN DIABETES ASSOCIATION, INC.
Arthur R. Colwell, Sr., M.D., *Chairman*
Dwight Ingle, Ph.D.
Maurice Krahl, Ph.D.
Rachmiel Levine, M.D.
Henry T. Ricketts, M.D.

Henry Rawle Geyelin
1883-1942

William C. Stadie, M.D., Philadelphia

H. Rawle Geyelin was born in Villanova, Pennsylvania, May 12, 1883. Unlike many of the sons of the old families who obtained their college and even preparatory education away from home, Geyelin received all of his formal education in Philadelphia. He prepared for college at the Haverford Grammar School and in 1902 entered the University of Pennsylvania in the combined course leading to an A.B. degree in 1906 and M.D. in 1909. Following graduation from medical school he served an internship in Philadelphia and was fortunate then to be able to have a period of study in Germany. Here were planted the seeds which stimulated his interest in clinical research, particularly along chemical lines.

Upon his return from abroad Geyelin moved to New York in 1912 to begin his graduate medical career at the Presbyterian Hospital. He was fortunate to have for his chiefs of medical service two men who were not only sympathetic but actively encouraged the new advances in clinical research which were beginning to develop in the metropolitan hospitals of this country. Theodore C. Janeway was Professor of Medicine at Columbia University when Geyelin began his postinternship work. Later, Warfield T. Longcope succeeded Janeway who had left to assume the Professorship of Medicine at Johns Hopkins University. New laboratory methods for the scientific study of clinical problems were being developed rapidly in Germany and both Janeway and Longcope were eager to apply them to clinical problems encountered in the medical service of the Presbyterian Hospital. Geyelin was called upon to undertake this task.

The difficulties of this undertaking are best appreciated when it is realized that Geyelin's general cultural background was about what was considered appropriate for a physician at that time. He had had little training in physics and chemistry in either high school or college and his knowledge of these fields was not appreciably increased during his medical training. The men in the city of New York who knew these fields well enough to be helpful to him were few indeed, and he was forced to rely mainly upon his own resources and diligent study of the literature. But to overcome these handicaps in fundamental scientific training he was filled with an ardent desire to master the new technics. He wanted to develop a critical judgment in applying them to clinical problems, and an ability to ask the right questions and frame the appropriate experiments by which they could be answered by the laboratory methods which were then available. In addition he had

the capacity for hard work. These attributes of character were more than enough to make up for his lack of specific knowledge in the chemical and metabolic fields.

At the beginning of his research work at the Presbyterian, Geyelin served as the Blumenthal Fellow in Medicine and he chose for his field the application of chemistry to problems of metabolism in disease. Geyelin's initial interest was the study of carbohydrate metabolism in thyroid disease, but he soon shifted to diabetes. At that time only cumbersome and laborious methods were available for blood or urine analysis. For example, the old Bertrand method was standard for blood sugar determinations. This required the withdrawal of 50 to 100 cc. of blood which was defibrinated by stirring in an evaporating dish as it was drawn from the vein. The blood was heated and a deproteinized filtrate prepared. Then followed the preparation of a cuprous oxide precipitate which had to be washed and weighed. Soon, however, Benedict, Folin and Van Slyke, among others, developed accurate and relatively simple new microchemical tools for the determination of various constituents of blood. Geyelin was ready for the application of these new methods to the many problems which presented themselves for study in the hospital wards. Meanwhile he had gathered around him a devoted group of younger men who were prepared to work under his direction.

Geyelin was determined that this new investigational approach should be made available for the study of patients in the general wards, in the outpatient clinics, and in private offices. This, indeed, was an ambitious scheme because it was the current opinion among the older men—who formulated the policies of the medical school and the clinic—that these difficult and laborious processes of research in clinical medicine could only be employed properly on selected patients in research institutions or in carefuly isolated metabolism wards. Geyelin, however, applied himself so assiduously to his new endeavor that within a few years he had established an efficiently functioning metabolic service staffed by a group of devoted young physicians. This service made possible the adequate study of a great variety of problems in patients who were being cared for by general nursing methods in the wards of the hospital. This enthusiasm for research in clinical medicine spread not only among the internes but to the students. The group at the Presbyterian Hospital, as in many similar ones, was constantly renewed by young men coming up through the ranks. From these men came the leaders in medical research and clinical advancement in the United States for the next half century.

A new era in medicine had begun in this country. The leaven, which had worked so well on the Continent and in England to stimulate advances in medical research, had been imported by American students who were fortunate enough to spend a year or two in study abroad. Within a short time the new point of view brought about a revolution in the organization of medical training here, influencing the medical schools as well as the hospitals. Geyelin was numbered among the pioneers who helped in the initiation of these changes.

Much of Geyelin's early work in diabetes preceded the advent of insulin. His best-known work was on the famous diabetic, C. K., who was considered an example of a totally diabetic individual. Geyelin studied him for many years. C. K. eventually went to the Russell Sage Institute in New York where methods for the determination of total metabolism were available. Much of the chemical data published in association with the total calorimetric work at the Russell Sage came from the laboratories of Geyelin at the Presbyterian Hospital.

Geyelin was one of the first to use insulin in juvenile diabetes. He published a carefully documented paper containing detailed metabolic studies on nine juvenile diabetics ranging from the age of two to fourteen. This was in August 1922 when very little insulin was available for research, and even less information was at hand to guide its therapeutic use. Geyelin had no idea how much insulin to use to offset the glycosuria on a given carbohydrate diet, and he had to feel his way with utmost caution in each individual. By stepwise increase of insulin dosage and carbohydrate intake he succeeded in arresting the downward course of the disease, achieving in each case a total food intake appropriate to the age requirement in calories for the individual. He demonstrated in every case a steady gain in weight and growth, and an increase in mental and physical vigor. By his caution and care in the use of the available insulin he was able to avoid any serious incidence of overdosage.

In essence Geyelin was engaged in a full-time academic career many years before medical schools recognized such specialization. But this academic career ended just before World War I. Sheer necessity compelled him to open an office for the practice of medicine. Here he achieved immediate success, but the onset of a succession of illnesses limited his activities. His last years were filled with pain and anxiety, but he wasted no time in self-pity or depression but kept on actively until his death in 1942.

The main contribution of Geyelin might be regarded as the demonstration that scientific procedures along chemical and other lines could be conducted with ac-

curacy and reliability on patients who were taken care of in the open wards. The influence of this work upon young physicians and upon students in stimulation of desire and interest to further research work in clinical medicine was incalculable.

During his life Geyelin was associated professionally in the following positions: at Presbyterian Hospital, Blumenthal Fellow in Medicine 1912-16, Assisting Visiting Physician 1915-21, Associate Attending Physician after 1921; at Babies' Hospital, Consulting Physician 1923-28, Assistant Visiting Physician 1928-32, Associate Attending Physician after 1932; at Vanderbilt Clinic, Chief of Medical Clinic 1918-19; at Columbia University, College of Physicians and Surgeons, Instructor in Clinical Pathology 1913-16, Associate in Clinical Pathology 1916-17, Associate in Medicine 1917-21, Assistant Clinical Professor of Medicine after 1921. He was also Consultant Specialist in Diseases of Metabolism at the United States Veterans' Hospital, Number 81, New York City, from 1924 to 1933.

Geyelin was a member of many scientific societies, among which were the American College of Physicians, the Association of American Physicians, the Harvey Society, the Interurban Clinical Club and many others. His clubs were the Century Association of New York and the University Barge Club of Philadelphia.

BOOK REVIEWS

ABC FOR DIABETICS, A MANUAL FOR PATIENTS. (ABC FUR ZUCKERKRANKE, EIN RATGEBER FUR DEN KRANKEN) *By Prof. Dr. F. Bertram, Hamburg, $1.00, pp. 84. 8th completely revised edition, Georg Thieme Verlag, Stuttgart, Germany, 1956.*

The new edition of Bertram's well-known manual for diabetic patients summarizes again in clear and simple form the essentials of etiology, symptomatology and therapy of diabetes mellitus. As in the previous editions the importance of dietary management is stressed and the value of oatmeal days, which have not found equal appreciation in the U.S.A., is emphasized. Insulin treatment and the technic of insulin injections is thoroughly described as well as the importance of exercise, hygiene and regularity in mode of living for the control of diabetes and the prevention of complications.

The American reader will be surprised, however, that some etiologic and therapeutic concepts are presented to the patient as factual information which at least in this country are considered to belong still in the field of investigation. Thus diabetes is divided into the commonly accepted insulin-deficiency type and into the contra-regulatory type which is said to be due to preponderance of the A cells and to hyperfunction of glucagon. The oral therapy with sulfonylurea compounds is presented as a distinct practical therapeutic progress useful particularly in the contraregulatory type. The author makes the rather bold statement that "there are several drugs which act in a large number of diabetic patients, unfortunately not in all, often even better than insulin and which offer in addition the advantage that they do not need to be injected." Accordingly, the chapter on dangers of the sulfonylureas minimizes their possible side effects and conveys the impression that the new drugs are safer than insulin, since they do not produce hypoglycemic reactions. The serious shortcoming of these drugs that they are ineffective in diabetic acidosis is not mentioned.

It would have been wiser, particularly in a manual for the laity, to indicate clearly which part of the information is based on secure and confirmed knowledge and which is speculative or hypothetical.

ESSAYS IN BIOCHEMISTRY. *Edited by Samuel Graff. $6.50, pp. 345, John Wiley & Sons, Inc., New York, 1956.*

This collection of twenty-five splendid essays was assembled to honor Hans Thacher Clarke on the occasion of his retirement as Professor of Biochemistry at the College of Physicians and Surgeons, Columbia University. The essays were written by Professor Clarke's former students and close academic associates, all of them distinguished leaders in the field of biochemistry. They cover a wide range of subjects, indicated by this partial list of topic headings: metabolic products of fungi; development of a plasma expander; conjugated proteins; thymine metabolism; steroid hormones; biochemistry of bacterial viruses; the nature of cancer; lipid metabolism; nitrogen-sparing effect of glucose; inositol; ferratin; biosynthesis of porphyrins; chemical structure of proteins; glycogen turnover; and the chemical basis of heredity determinants. The discussions constitute a critical survey of the present status of many major problems in several closely allied fields of biochemical research, and offer provocative speculations on probable future trends. The concise well-written essays reflect the high standards of scientific scholarship set by Professor Clarke.

ABSTRACTS

Abdala, Alberto (Universidad Nacional de Córdoba, Córdoba, Argentina): DIABETIC GLOMERULOSCLEROSIS, GLOMERULAR HYALINOSIS, AND HYALINE FIBRINOID LESIONS. Rev. conf. med. panamericana 3:171-76, May 1956.

A comparative study is made of the pathologic findings in the kidneys of known diabetics, of patients with glomerulonephritis in the acute, subacute, and chronic stages, and also of patients with nephrosclerosis. The Kimmelstiel-Wilson lesions were found only in the diabetic kidneys and, in one of them were associated with hyaline fibrinoid lesions. (Spanish)

Becker, W. H.; Buddecke, E.; and Mueller, H. (Chirurgische Klinik und Physiologisch-Chemische Inst., Justus-Liebig-Hochschule Giessen, Germany): EFFECT OF BZ-55 ON THE BLOOD SUGAR OF THE PANCREATECTOMIZED DOG. Klin. Wchschr. 34:920-21, 1956.

Totally depancreatized dogs in which the pyloric region of the stomach and the duodenum had also been resected were given BZ-55 intravenously in doses varying from 0.8 to 1.5 gm. per kg. in 10 or 20 per cent solution of its sodium or ammonium salt. The diabetes of these animals had been controlled by depot-insulin (1-1.5 units/kg./day). Insulin was discontinued twenty-four hours before the injection of BZ-55. At that time the fasting blood sugar was markedly elevated (300-500 mg. per cent). In each instance the administration of BZ-55 was followed by a significant decrease of the hyperglycemia which lasted for about six to eight hours and amounted to about 15 to 40 per cent of the initial value, or an absolute decrease from 100 to 150 mg. per cent. This decrease was, however, smaller than that in partially depancreatized, nondiabetic dogs treated with BZ-55 in the same fashion; in these it amounted to 50 per cent or more of the initial value, and severe hypoglycemic reactions could be induced. The authors conclude from these experiments that BZ-55 does not act, at least not exclusively, upon the islet-cell system of the pancreas. They postulate that the drug inhibits peripheral enzymatic degradation of insulin and thus permits small amounts of exogenous insulin which might still be present in the depancreatized dogs to become effective again; likewise, it may enhance the action of endogenous insulin, as demonstrated in the partially depancreatized dogs. Control experiments in which insulin is withheld from depancreatized dogs for more than twenty-four hours are not reported.

Behrer, M. Remsen; Goldring, David; and Hartman, Alexis F. (Dept. of Pediat., Washington Univ. Sch. of Med., and St. Louis Children's Hosp., St. Louis, Mo.): THE TREATMENT OF DIABETIC ACIDOSIS: COMPARISON OF TREATMENT REGIMES WITH AND WITHOUT PARENTERAL POTASSIUM. J. Pediat. 49:141-64, August 1956.

The blood chemical determinations and serial electrocardiograms of fifteen juvenile diabetics in moderate to extreme acidosis are reported. These patients were treated according to the Hartman regime and did not receive added potassium. The clinical syndrome of hypokalemia was not observed. No parenteral potassium therapy was necessary, since all patients responded rapidly and were taking either liquid or soft diets in the first twenty-four hours and were ambulatory in forty-eight hours.

A group of seven juvenile diabetics in severe or extreme acidosis received added parenteral potassium initially and were studied in a similar manner. The serum potassium levels were followed and showed the usual early depressions as in Group I, but there seemed to be no correlation with the clinical state of the patient or the serial electrocardiographic findings.

No significant differences could be seen in the electrocardiograms of Group I and Group II. There seems to be a reasonable doubt that the electrocardiographic changes can be attributed solely to hypokalemia; they may be the result of other factors in such a disturbed metabolic state as diabetic acidosis.

Bencosme, Sergio A.; and Lazarus, Sydney S. (Dept. of Pathol., Queen's Univ. Faculty of Med., Kingston, Ontario, Canada): THE PANCREAS OF CORTISONE-TREATED RABBITS; PATHOGENIC STUDY. A.M.A. Arch. Path. 62:285-95, October 1956.

An attempt is made to determine the pathogenesis of the diffuse ductular hyperplasia which was reported to occur in cortisone-treated rabbits. It is found that definite lesions are observed as early as eight days and that neither antibiotics nor vitamin supplements (A, B, C or D) influences the morphologic pattern. The pancreas from animals with alloxan diabetes or from rabbits subjected to partial or complete starvation does not present the ductular change associated with cortisone therapy. However, definite similarities are found between the morphology of the duct-ligated and that of the cortisone-treated pancreas. This similarity is taken to indicate that, in the latter, the lesion is also obstructive in nature, possibly caused by cortisone-induced changes in the

viscosity of the secretory material. A possible role of infection, or of a direct response of ductular epithelium to cortisone, cannot be ruled out. The differences in the appearance of the duct-ligated and of the cortisone-treated pancreas are thought to be due to differences in the site of obstruction. In the latter, the obstruction may be either at or above the level of the intralobular ducts; whereas in the former, the main pancreatic duct is obstructed. Furthermore, it is thought that the obstruction in the cortisone-treated animal is probably progressive and possibly intermittent. This would account for the presence of lesions of varying degrees in a single section of the cortisone-treated pancreas, as compared with the uniformity of the appearance of the duct-ligated pancreas at any given stage.

Beringer, A.; Burkl, W.; and Steinhardt, O. (I. Med. Clin. of the Univ. of Vienna, Austria): FUNCTION OF ALPHA CELLS OF THE PANCREAS. Wien. klin. Wchnschr. *68*:301-04, April 13, 1956.

The alpha to beta-cell ratio in the pancreatic islets of alloxan diabetic rabbits is very high because most of the beta cells are destroyed. If the diabetic hyperglycemia were caused not by the absence of the beta-cell hormone, insulin, but by the preponderance of the alpha-cell hormone, glucagon, one should expect a decrease of the liver glycogen in alloxan diabetes. Fasting rabbits with mild alloxan diabetes, however, store more glycogen in the liver than do fasting control animals, in spite of the fact that the alpha-cell ratio is higher in the alloxanized group. If given insulin and glucose, rabbits with severe alloxan diabetes store the same amount of glycogen in the liver as do normal rabbits receiving the same amount of glucose without insulin. When insulin is withheld, the liver glycogen of the animals with severe alloxan diabetes declines at the same rate as that of the normal control animals. Operative removal of the whole alpha-cell system (extirpation of pancreas, stomach, and small intestine) does not affect the insulin sensitivity, nor does it decrease the hyperglycemia of rabbits with severe alloxan diabetes. The authors feel that their experiments are evidence against the hypothesis that the alpha cells and their hormone play an essential role in the origin of diabetic hyperglycemia.

Berlinger, A.; and Lindner, A. (I. Med. Clin. of the Univ. of Vienna, Austria): MECHANISM OF ACTION OF THE BLOOD SUGAR LOWERING SULFONAMIDES. Wien. klin. Wchnschr. *68*:316-22, April 20, 1956.

Compared with the liver glycogen of untreated fasted animals (0.55 per cent) 2.5 gm. per kg. of N_1-sulfanilyl-N-butylcarbamide (BZ-55) given orally to fasting rabbits caused an increase in liver glycogen of 2.3 per cent. The glycogen content of the skeletal muscle remained unchanged. The increase of liver glycogen after BZ-55 was similar to the effect of a continuous intravenous infusion of small amounts of glucose; yet after BZ-55, the blood sugar did not increase, and the better glucose assimilation in the liver occurred in spite of a fall of the blood sugar. In rabbits with severe alloxan diabetes BZ-55 did not cause a lowering of the blood sugar; the presence of insulin seems to be necessary to produce this effect. In human diabetes, prolonged medication of BZ-55 seems to improve peripheral glucose utilization, as indicated by the finding of increased capillary-venous blood sugar differences.

Brennan, C. F.; Malone, R. G. S.; and Weaver, J. A. (Royal Victoria Hosp., Queen's Univ., Belfast): PITUITARY NECROSIS IN DIABETES MELLITUS. Lancet *2*:12-16, July 7, 1956.

This article presents clinical and pathological findings of five diabetics who had sustained necrosis of the anterior pituitary. Necropsy showed that such necrosis was found to occur more frequently in diabetics than in nondiabetics.

Broide, Lázaro (Havana, Cuba): TEN RULES FOR THE ADMINISTRATION OF INSULIN IN DIABETIC COMA. Rev. méd. cubana *67*:250-57, March 1956.

The rules for the administration of insulin in diabetic coma are summarized: Administration should be early, continuous and regulated by the clinical response and the laboratory. The initial dose should be large, and the subsequent doses should be given at intervals of fifteen to thirty minutes, depending upon the seriousness of the coma. Most of the total dosage of insulin is given in the first three hours, and there is no maximal dose to produce insulin resistance. (Spanish)

Burdon, J. F. (Paignton, England): PENICILLIN V AND DIABETES. Brit. M. J. *2*:997, Oct. 27, 1956.

The author describes a severe diabetic with pneumonia who responded well to initial penicillin therapy by injection. Following the substitution of oral penicillin therapy, the patient's diabetic control rapidly deteriorated. On cessation of oral penicillin medication, the patient returned to his normal stable condition within twelve hours. The author suggests that the oral penicillin might be the cause of loss of control of the diabetes and warns other physicians to be on the lookout for similar situations.

Constam, G. R. (Med. Policlinic, Univ. of Zurich, Switzerland): MODE OF ACTION AND INDICATION FOR

BZ-55 IN THE TREATMENT OF DIABETES MELLITUS. Méd. et Hyg. *14*:122, March 30, 1956.

The pancreas seems to be the point of action for the hypoglycemic sulfonamides. Their activity apparently is connected with the presence of insulin. Possibly they inhibit an insulinase, but they might also increase the production of the secretion of insulin. An inhibitory action on glucagon is unlikely. BZ-55 may be tried in the treatment of asthenic diabetes which started after the age of forty-five and had insulin for less than five years. It is contraindicated in the treatment of infantile or juvenile diabetes and of asthenic diabetes which began before the age of forty-five. In cases of relative insulin resistance, BZ-55 may be tried. For the substitution of insulin by BZ-55, patients must stay under hospital observation for several days. Diet is fundamental for a successful treatment with BZ-55.

Dury, Muriel; and Dury, Abraham (Dorn Lab. for Med. Res., Bradford Hosp., Bradford, Pa.): EFFECTS OF ASCORBIC ACID PRETREATMENT ON THE RESPONSE OF BLOOD GLUCOSE AND ADRENAL CHOLESTEROL IN THE INTACT RAT TO INSULIN. Endocrinology *58*:671-74, May 1956.

Pretreatment of intact rats with ascorbic acid, causing blood levels six to eight times greater than controls, failed to modify insulin sensitivity or affect liver glycogen or postprandial blood glucose concentrations.

Editorial: INSULIN ZINC SUSPENSIONS. Lancet *3*:1032-33, Nov. 17, 1956.

The author describes the development of and current experience with insulin zinc suspensions (Lente insulins).

Editorial: THE DIABETIC FOOT. Brit. M. J. *2*:1047, Nov. 3, 1956.

The author refers to recent literature concerning the enlightened care and treatment of diabetic foot and its complications.

Forsyth, Constance C.; and Payne, W. W. (The Hospital for Sick Children, Great Ormond St., London, England): FREE DIETS IN THE TREATMENT OF DIABETIC CHILDREN. Arch. Dis. Childhood *31*:245-53, August 1956.

The authors review their findings on one hundred juvenile diabetic patients observed for varying periods of time and report their findings in regard to the incidence of degenerative complications in those who have reached adult life. In their experience, the use of "free diets" has improved the degree of control in these patients, and their over-all results compare favorably with those of most clinics. The authors point out the importance of maintaining good control to avoid a higher incidence of retinitis.

In the opinion of the abstracter, the introduction of the term "free diet" has resulted in considerable confusion since the term is interpreted differently by different people. It is not the dietary plan used but rather the degree of control attained by the over-all management that is important in preventing degenerative complications.

Ginsburg, Jean; and Paton, A. (Sherrington Sch. of Physiol.; St. Thomas's Hosp., London, England): EFFECTS OF INSULIN AFTER ADRENALECTOMY. Lancet *2*:491-94, Sept. 8, 1956.

This article presents the observations on twelve patients who underwent adrenalectomy for carcinomatosis or malignant hypertension. Intravenous insulin tests produced hypoglycemia with symptoms and the usual return of the blood sugar to the fasting levels. It is concluded that adrenalin is not solely responsible for the restoration of the normal level of circulating glucose after insulin hypoglycemia.

Hackedorn, Howard M. (Seattle, Wash.): ORAL HYPOGLYCEMIC AGENTS FOR TREATMENT OF DIABETES MELLITUS. Northwest Med. *55*:965-67, September 1956.

Bearing in mind the clinician who is not primarily concerned with diabetes, the author reviews current thoughts on actions, uses, effects and dangers of the oral sulfonylureas in the treatment of diabetes.

Hazlewood, Robert L.; Bennett, Leslie L.; and Nelson, Marjorie (Dept. of Physiol. and the Inst. of Exper. Biol., Univ. of California, Berkeley, California): EFFECT OF PANTOTHENIC ACID DEFICIENCY ON INSULIN SENSITIVITY AND RESPONSE TO ACTH OF INTACT AND DIABETIC RATS. Endocrinology *58*:427-34, April 1956.

Insulin sensitivity in either intact or partially depancreatized diabetic rats was found to vary with the duration of pantothenic acid deficiency. In the early stages, there was evidence of insulin resistance; in the later stages, increased sensitivity. This finding correlated with findings of adrenal hyperfunction early in the deficiency state and of adrenal hypofunction after a longer period of pantothenic acid deficiency.

Irvine, R. E.; and Rowell, N. R. (Med. Registrars, Royal Victoria Infirmary, Newcastle-upon-Tyne, England): COMA IN DIABETES WITH RENAL FAILURE. Lancet *2*:1025-26, Nov. 17, 1956.

The authors describe a case of recurrent coma in a patient with renal failure due to diabetic nephropathy and chronic pyelonephritis. The main features were severe acidosis and hyperglycemia without ketonemia and ketonuria. The possible explanations of these findings are discussed, with special reference to the impor-

tance of testing the blood for ketones in any case of apparent diabetic coma without ketonuria.

Jackson, W. P. U.; and Woolf, N. (Endocrine Clin. & Dept. of Path., Groote Schuur Hosp. & Univ. of Cape Town, South Africa): THE NATURAL HISTORY OF PREDIABETES. New England J. Med. *255*:1183-85, Dec. 20, 1956.

A woman developed diabetes mellitus which had not been apparent during ten pregnancies. Because of the size of her earlier infants, a glucose tolerance test had been done but failed to demonstrate diabetes. Sections from a stillbirth two years prior to her last pregnancy were reviewed and found to show marked pancreatic islet hypertrophy. Because of suspicion of a prediabetic state, a section before term was done, with survival of a thirteen-pound child. The authors suggest prediabetes might be considered as a possible cause for any unexplained stillbirth.

Jersild, M. (Hvidore Hospital, Copenhagen, Denmark): INSULIN ZINC SUSPENSIONS: FOUR YEARS' EXPERIENCE. Lancet *2*:1009-13, Nov. 17, 1956.

The author reviews the diabetic control of 1,000 patients who were treated with Lente insulins, 90 per cent of whom were admitted to his hospital for the initial adjustment. Follow-up after an average period of eighteen months showed that 82 per cent of the patients who had originally been controlled on one injection daily had been mantained on this regimen. Of the group originally receiving two injections daily, 19 per cent had later been transferred to one injection daily. Urinary excretion of sugar was greatest in patients without retinopathy or nephropathy; but the blood sugar level of patients with and without these complications was, on the average, the same. Treatment with preparations of insulin zinc suspension (Lente insulin) led to satisfactory results, even in most of the cases in which control had previously been difficult.

Joos, Thad H.; and Johnston, Joseph A. (Dept. of Pediat., Henry Ford Hosp., Detroit, Mich.): A LONG-TERM EVALUATION OF THE JUVENILE DIABETIC. J. Pediat. *50*:133-37, February 1957.

In the regulation of diabetes mellitus in children, control is of the utmost importance when prevention of serious vascular complications is considered. Retinopathy and albuminuria are the most frequently encountered vascular complications. Complications of this sort first become detectable at an average of eleven years and eight months after the initial diagnosis. There is increased need for insulin and calories as growth progresses. A peak is reached at puberty, followed by a drop; then stabilization of both requirements takes place.

King, John W.; and Hainline, Adrian, Jr. (Dept. of Clin. Path., Cleveland Clinic, Cleveland, O.): COMMERCIAL GLUCOSE OXIDASE PREPARATIONS FOR THE DETECTION OF GLUCOSE IN URINE. Cleveland Clin. Quart. *23*:212-15, July 1956.

In the testing of 1,000 routine urine specimens, two commercial glucose oxidase preparations were more sensitive to glucose in the urine than the conventional Benedict's test. The glucose oxidase prepararions are not as convenient as Benedict's test for large-scale testing; but if their high sensitivity is taken into consideration, they are excellent products for use in the office laboratory, at the bedside, or by the diabetic patient himself. These reagents also are useful reference tests in the determination of the nature of copper-reducing, nonglucose substances in urine because they are highly specific for glucose.

Klein, R.; and Laron, Z. (Dept. of Pediat., Univ. of Pittsburgh; and Children's Hosp. of Pittsburgh, Pittsburgh, Pa.): CURRENT PROBLEMS IN DIABETES. Pediatrics *18*:983-96, December 1956.

The authors discuss in an interesting review article the relationship of adrenal cortical function to vascular changes. They also review and discuss recent studies of sulfonylureas in treatment of diabetes.

Lawrence, R. D. (London, W. 1, England): ENZYME TEST FOR GLYCOSURIA. Brit. M. J. *2*:1058, Nov. 3, 1956.

The author comments on the proper procedure for performing the Benedict qualitative test for urine sugar, which requires 5 ml. of the reagent for eight drops of added urine.

Munro, I. B.; and Murray, D. (Dept. Metabolic Diseases, Victoria Infirmary, Glasgow, Scotland): EFFECT OF CARBUTAMIDE ON SERUM-CHOLESTEROL LEVEL IN DIABETES MELLITUS. Lancet *2*:1083-84, Nov. 24, 1956.

The authors describe the effect of carbutamide on serum cholesterol in two diabetic patients. A drop in serum cholesterol was noted following this therapy. Two normal subjects given the same dosage of carbutamide failed to reveal a similar change in cholesterol levels.

Nissel, W.; and Dworschak, W. (I. Med. Dept. of the Gen. Polyclinic of Vienna, Austria): INFLUENCE OF CARBOHYDRATE METABOLISM BY DIAMOX. Wien. klin. Wchnschr. *68*:264-67, March 30, 1956.

The blood sugar curve was not changed significantly in the double glucose test (Staub-Traugott or Exton-Rose test) when 500 mg. Diamox (2-acetyl-amino-1, 3, 4-thiodiazol-5-sulfonamide) was administered to twenty-five nondiabetic subjects. In nine out of thirteen dia-

betics, however, the same dose of Diamox lowered the blood sugar curve and decreased the glycosuria. It appears, therefore, that Diamox has a favorable influence upon the carbohydrate tolerance of the diabetic and can be used in diabetes without danger. Acidosis was not observed.

Oakley, Wilfrid; Catterall, R. C. F.; and Martin, M. Mencer (King's Coll. Hosp., London, England): AETIOLOGY AND MANAGEMENT OF LESIONS OF THE FEET IN DIABETES. Brit. M. J. *2*:953-57, Oct. 27, 1956.

The authors report the incidence of foot lesions in the diabetics attending their clinic as well as sex, age, and duration of diabetes. They draw attention to the importance of neuropathy, alone and in association with ischemia, as a factor in the production of localized ulceration and gangrene. The treatment of lesions due to neuropathy and ischemia is described, and the value of such operations as removal of all five toes and amputation below the knee is emphasized. A method of protection of heels in patients confined to bed is described and illustrated. Reasons are given for doubting the validity of the theory that the high incidence of so-called vascular lesions of the feet in diabetes is evidence that this disease by itself commonly causes peripheral occlusive vascular disease.

Paulson, Walter O. (Midelfart Clin., Eau Claire, Wisc.): "PRECLINICAL" DIABETES MELLITUS AND PREGNANCY. Am. J. Obst. & Gynec. *72*:1152-55, November 1956.

A clinical case showing the relationship between preclinical diabetes mellitus and pregnancy is described. It discloses an increasing birth weight with each pregnancy. Death and maceration of the fetus were seen in the fourth pregnancy. Clinical diabetes mellitus developed five years after the last pregnancy. Some of the experimental and clinical literature on this subject is reviewed in this article.

Portuondo de Castro, J. M.; Casares Escarra, Enrique; Goicoechea y Quirós, Pilar; Muñiz Cano, Reinaldo; Justiniani y Longa, Federico; and Quesada y Ramírez, Emilio de (Cátedra de Patologia Médica con su Clinica Havana, Cuba); OBSERVATIONS ON FIFTY CASES OF DIABETIC COMA IN THE UNIVERSITY HOSPITAL "GENERAL CALIXTO GARCIA." Arch. Hosp. Universitario 7:427-35, November-December, 1955.

The mortality rates were from 27 to 33 per cent, which, it is explained, were due to the critical condition of the patients when they were admitted. In 28 per cent of the cases, the existence of diabetes was unknown; in 70 per cent, omission of insulin was the cause of the diabetic coma. (Spanish)

Randle, P. J.; and Young, F. G. (Dept. of Biochem., Univ. of Cambridge, England): THE INFLUENCE OF PITUITARY GROWTH HORMONE ON PLASMA INSULIN ACTIVITY. J. Endocrinol. *13*:335-48, April 1956.

The increased insulin activity of plasma from growth hormone treated intact cats may be attributed to an increase in insulin content of the plasma. Growth hormone did not appear to influence plasma insulin activity of intact rats. In the cats, the increased insulin activity appears to result from an increased rate of insulin secretion by the islets in response to the growth hormone rather than from a depression of the rate of disappearance of insulin by growth hormone. The reasons for such an effect are not clear.

Reynall, P. C.; and Spray, G. H. (Nuffield Dept. of Clin. Med., Univ. of Oxford, England): THE ABSORPTION OF GLUCOSE BY THE INTACT RAT. J. Physiol. *134*:531-37, Dec. 28, 1956.

The effect of varying doses of glucose on the rates of glucose absorption by the stomach and small intestine has been studied in the intact male Wistar rat (200-400 gm. body weight). The stomach absorbs glucose at a rate independent of the glucose concentrations tested. Over the range of glucose loads studied (170-635 mg.) the rate of absorption by the small intestine increased with increasing loads. Even at the highest loads tested, most of the glucose was still absorbed by the first half of the small intestine. It has not proved possible to saturate the absorptive capacity of the small intestine of the rat for glucose. The introduction of hypertonic glucose solutions into the stomach results in slowed emptying into the small intestine at a rate which is less than its maximum absorptive capacity.

Rosten, B. M. D. (Hanworth, Middlesex, England): ENZYME TESTS FOR GLYCOSURIA. (CORRESPONDENCE). Brit. M. J. *2*:940, Oct. 20, 1956.

The author compares the advantages and drawbacks of "Clinistix" (Ames) and Tes-Tape (Urine Sugar Analysis Paper, Lilly) in the detection of glycosuria.

Rosten, B. M. D. (Hanworth, Middlesex, England): ENZYME TEST FOR GLYCOSURIA (CORRESPONDENCE). Brit. M. J. *2*:1238, Nov. 24, 1956.

The author clarifies a statement in an article published by him and reviewed subsequently by Dr. R. D. Lawrence (*Brit. M. J.* Nov. 3, 1956, page 1058).

Scheffler, H.; and Hagen, H. (Medizinische Klinik u. Diabetikerheim des Wald Krankenhauses, Zeven, Hann, Germany): GROWTH, HEREDITY AND AGE OF MANIFESTATION OF DIABETES MELLITUS IN CHILDREN AND JUVENILES. Klin. Wchnschr. *34*:793-96, 1956.

No abnormalities in growth and development were found in a group of 118 juvenile diabetics aged four to twenty years (fifty-eight boys and sixty girls). It appears that, nowadays, insulin control permits the diabetic child to develop at the same rate as the nondiabetic. In 33 per cent of the group, some other sibling had diabetes. It is believed, however, that in juvenile diabetes the incidence of heredity is higher, since no complete checks could be made, and diabetes in the parent generation sometimes manifests itself much later than in the children. The onset of the diabetes was during puberty or prepuberty in the majority of cases. No evidence was found for another peak of incidence between ages five and seven.

Schneeweiss, J. (Städt Krankenhauses, Berlin-Hohengatow, Germany): DIABETES AND GALL-BLADDER DISEASE. Deutsche med. Wchnschr. *81*:1356-58, Aug. 24, 1956.

Statistics on 3,788 diabetics (63 per cent female) hospitalized between July 1, 1946, and June 30, 1955, show that, assuming similar nutritional status, diabetics have no higher incidence of disease of the biliary system than nondiabetic patients. This finding was confirmed by age-matched autopsies of 300 diabetics and the same number of nondiabetics. However, nutritional changes (as demonstrated in Berlin when normal food supply was restored) are important: There is a correlation between the incidence of biliary disease and the fat content of the food. This may explain the higher incidence of biliary disease among diabetics previously reported by other authors at a time when a diabetic diet had a higher fat content than is now the case.

Sheridan, E. Paul; and Cullen, Richard C. (Denver, Colo.): MANAGEMENT OF DIABETES DURING PREGNANCY. Rocky Mountain M. J. *53*:721-27, August 1956.

A general outline of the medical management of diabetics during pregnancy is presented, with the realization that many questions still remain unanswered. The authors are convinced that the most important factor in reducing fetal mortality is good diabetic control, regardless of whether hormones are used or pregnancies terminated. A preliminary report of thirty-six viable diabetic pregnancies is presented, with what is believed to be a satisfactory fetal salvage rate of 89 per cent.

Shuffstall, Richard M. (Pennsylvania Hospital, Philadelphia, Pa.): THE PATHOLOGY OF DEGENERATIVE MANIFESTATIONS OF DIABETES MELLITUS. M. Clin. North America *39*:1693-1700, November 1955.

This is a pathological description of the vascular changes occurring in diabetes mellitus. A discussion and description of the atherosclerotic process, including importance of lipid metabolism, is presented. Arteriolar sclerosis of the small peripheral arteries is also described. The importance of the pathological changes of capillaries in diabetes is emphasized, especially in the changes that occur in the kidneys and in the retina. It is pointed out that capillary changes are not confined only to the renal glomeruli and the retina but that the hyalinization of the islets of Langerhans is thought by some to represent the same process. Capillary changes have also been noted in the conjunctiva and the nail beds. There is also evidence accumulating to include degenerative vascular changes in the vascular system, although these changes are not definite as yet. Pathological description of lens opacities is also given, and the various histological changes noted in the nerves associated with peripheral nerve injury found in diabetes are also discussed, as are spinal cord changes.

Shuman, Charles R.; Kemp, Robert L.; Coyne, Richard; and Wohl, Michael G. (Temple Univ. Hosp. and Metabolic Div. of Philadelphia Gen. Hosp., Philadelphia, Pa.): CLINICAL USE OF SORBITOL AS A SWEETENING AGENT IN DIABETES MELLITUS. Am. J. Clin. Nutrition *4*:61-67, January-February 1956.

The effects of feeding ice cream sweetened with the test substance sorbitol were noted on the blood sugars of mild and other more severe diabetics. Sorbitol, a sugar alcohol, may be regarded as an available carbohydrate which is metabolized through frutose or glucose to glycogen. The ice cream servings containing 36 gm. of sorbitol did not significantly alter the diurnal blood glucose values of mild and moderately severe diabetic patients.

Smith, Beverly Chew (New York, N. Y.): A TWENTY YEAR FOLLOW-UP IN FIFTY BELOW-KNEE AMPUTATIONS FOR GANGRENE IN DIABETICS. Surg. Gynec. & Obst. *103*:625-30, November 1956.

This abstract presents the technic for amputation below the knee with minimal trauma to tissues. An analysis of the survival periods and suitability of the stump for prostheses is favorable when compared with midthigh amputations.

Smith, M. J. H.; and Taylor, K. W. (Dept. Chemical Path., King's Coll. Hosp. Med. Sch., Denmark Hill, London, England): BLOOD PYRUVATE AND α-KETOGLUTARATE IN NORMAL AND DIABETIC SUBJECTS. Brit. M. J. *2*:1035-38, Nov. 3, 1956.

The authors report that normal blood concentrations of pyruvate and alpha-ketoglutarate were found in ambulatory diabetic patients in blood specimens collected either three hours after eating or after an overnight fast. When diabetic patients were divided into two main clin-

ical types, the administration of oral glucose caused a significant rise in blood pyruvate in the "obese group" at one hour similar to that found to occur in normal subjects, but not in the "thin group." Some of the implications of these results are discussed.

Spencer, A. G.; and Morgans, M. E. (Med. Unit., Univ. Coll. Hosp., Med. Sch., London, England): LENTE INSULINS: FOUR YEARS' EXPERIENCE. Lancet *2*:1013-17, Nov. 17, 1956.

The authors treated 200 diabetic patients with Lente insulin and observed them for from six months to four years. The diabetes was controlled satisfactorily in 83 per cent of the patients on transfer to a single daily injection of Lente insulin, and in 36 per cent the control was improved.

Tomizawa, Henry H.; Nutley, Mary L.; Narahara, Hiromichi T.; and Williams, Robert H. (Dept. of Med., Univ. of Washington, Sch. of Med., Seattle, Wash.): THE MODE OF INACTIVATION OF INSULIN BY RAT LIVER EXTRACTS. J. Biol. Chem. *214*:285-94, May 1955.

Evidence is presented which indicates to the authors that insulin is probably proteolytically inactivated by rat liver extract at *p*H 7.5.

Törnblom, Nils (Med. Clin. and Central Lab., Univ. Hosp., Upsala, Sweden): ADMINISTRATION OF DDD (2, 2 - BIS (PARACHLOROPHENYL) - 1, 1 - DICHLOROETHANE) TO DIABETICS WITH HYALINE VASCULAR CHANGES AND HYPERPOLYSACCHARIDEMIA. A PRELIMINARY REPORT. Acta. med. scandinav. *154*:83-89, 1956.

Two diabetic patients with pronounced signs of hyaline vascular changes and hyperpolysaccharidemia have for a period of twelve months been given DDD (2, 2-bis [parachlorophenyl]-1, 1-dichloroethane) with the object of depressing the adrenal cortical function. Signs of serious hepatic damage were not observed. In one case, the serum polysaccharide level declined and the insulin requirement diminished. In the other case, the results were similar but less accentuated.

Tower, Donald B.; Peters, Edmund L.; and Pogorelskin, Milton A. (Natl. Inst. of Neurological Dis. and Blindness, Bethesda, Md.): NATURE AND SIGNIFICANCE OF PENTOSURIA IN NEUROMUSCULAR DISEASE. Neurology *6*:37-49, January 1956, concluding *6*:125-42, February 1956.

Twenty-four-hour urine excretions for free aldopentoses were studied in 230 specimens from fifty-five patients with and without muscle disease while on a fruit-free diet. Identities of the pentoses excreted by twenty-nine two-dimensional paper chromatograms of urines of eighteen patients were noted. Eight carbohydrate compounds were identified in urine specimens. The patients with muscular dystrophy, myotonia dystrophica, myasthenia gravis, and muscular atrophy excreted significantly higher levels of free aldopentoses per 24 hours per kilogram of body weight (6.6 μM for adults, 9.6 μM for children) than patients without muscle disease (3.6 μM for adults, 5.5 μM for children). Present evidence suggests that the results of the disease process contribute to the above findings rather than to an underlying biochemical lesion causing the disease.

Tunbridge, R. E.; and Paley, R. G. (Univ. Dept. of Med. General Infirmary, Leeds, England): INSULIN ZINC SUSPENSIONS. Lancet *2*:1161, Dec. 1, 1956.

The authors review their experience with the Lente insulins and describe their advantages and limitations. They caution that the dream of a single daily dose of insulin for all diabetes is not possible with the present-day insulins without the risk of unnecessary hazards.

Walker, G.; Leese, W. L. B.; and Nabarro, J. D. N. (Diabetic Clin., Middlesex Hosp., London, England): HYPOGLYCAEMIC SULPHONAMIDES IN TREATMENT OF DIABETES. Brit. M. J. *2*:451-52, Aug. 25, 1956.

The authors report on the use of BZ-55 in twenty-four stable middle-aged diabetics. Satisfactory reduction of the blood sugar was obtained in twenty-three, but two had to be taken off the drug because of a generalized rash.

Walker, Joan B. (*Correspondence*) (Leicester, England): BZ-55 IN DIABETES. Brit. M. J. *2*:657, Sept. 15, 1956.

The author comments on the progress of her investigation of the use of BZ-55 in thirty-four diabetics previously reported upon.

Williams, Robert H. (Dept. of Med., Univ. of Washington, Sch. of Med., Seattle, Wash.): INSULIN DISTRIBUTION AND DEGRADATION. Metabolism 5:128-37, March 1956.

Intravenous insulin-I^{131} is rapidly distributed throughout the body, with the highest concentration appearing in the liver and kidneys. It seems to be firmly fixed in tissue having characteristic loci in intracellular components. Insulin is rapidly degraded in the body by at least two factors—one heat stable and one heat labile. Substances are referred to that may be substrates for the heat-labile factor. Degradation is prevented by other substances, including an unidentified tissue fraction called "insulinase-inhibitor."

Wirts, C. Wilmer; Smith, Forrest M., Jr.; and Cooper, Donald W. (Jefferson Med. Coll.; Pennsylvania Hosp., Philadelphia, Pa.): THE MANAGEMENT OF COEXISTING

DIABETES AND PANCREATITIS. M. Clin. North America 39:1655-64, November 1955.

This article deals chiefly with a description of the etiology, pathology, symptoms and treatment of acute and chronic pancreatitis, with presentation of cases involving each type. The association of diabetes mellitus, which is more common, with the chronic phase of pancreatitis is mentioned; and the control of the diabetic state with insulin and diet along with the treatment of the pancreatitis is stressed.

Wolf, H. J.; and Preiss, H. (Med. Dept., Municipal Hosp., Bielefeld, Germany): EXPERIENCES WITH FAT FREE DIETS IN DIABETES MELLITUS. Deutsche med. Wchnschr. 81:514-15, April 6, 1956.

The influence of a fat-free diet upon hyperglycemia and glycosuria was studied in sixty diabetic patients, of whom thirty-three were treated with insulin and diet and twenty-seven with dietary restriction alone. The caloric content of the diet remained unchanged, but the fat content was altered in such a way that the usual amount of fat was given during an initial period of eight to ten days, no fat at all was permitted during a second period of eighteen days, and small amounts of fat were given during a third period of eight to ten days. About two days after fat withdrawal, a significant decrease in hyperglycemia and glycosuria was observed. This was independent of insulin dosage, duration of the disease, and sex or age of the patients. When fat was added, hyperglycemia and glycosuria increased proportionately to the amount of fat. In eight cases, the fat-free diet seemed to have a beneficial effect on ulcerative lesions of the legs.

Wolff, Frederick W.; and Stewart, G. A. (Whittington Hosp., London & Biological Control Labs., Wellcome Foundation, London, England): RESPONSIVENESS TO ORAL HYPOGLYCAEMIC AGENT. Lancet 2:628, Sept. 22, 1956.

The authors comment on the problem of devising a method to anticipate any particular patient's response to an oral hypoglycemic agent and cite their experience to date with this problem.

Wyman, A. L. (London, W. 6, England): LESIONS OF THE FEET IN DIABETES (CORRESPONDENCE). Brit. M. J. 2:1239, Nov. 24, 1956.

The author states his views concerning the etiology of diabetic foot lesions.

Young, F. G. (Univ. of Cambridge, Cambridge, England): HYPOGLYCAEMIC AND ANTIDIABETIC SULPHONAMIDES. Brit. M. J. 2:431-32, Aug. 25, 1956.

The author reviews the history and present status of hypoglycemic and antidiabetic sulfonamides and the various theories relative to their modes of action. It is concluded that, at present, there is no simple solution at hand to the problem of the mechanism of action of these drugs.

Young, Keith, R.; and Clancy, Carl F. (Pennsylvania Hospital; Jefferson Med. Coll., Philadelphia, Pa.): URINARY TRACT INFECTIONS COMPLICATING DIABETES MELLITUS. M. Clin. North America 39:1665-70, November 1955.

The authors studied the autopsy records of the Ayer Clinical Laboratory of the Pennsylvania Hospital as to urinary tract infections of all types for a ten-year period. Of the 1,640 autopsies reported, seventy-two were diabetics. The incidence of urinary tract infections ranged between 59 and 70 per cent of the group as a whole; the over-all percentage for the diabetics was 42 per cent. Clinical studies of catheterized urines of sixty-two female diabetics were also performed; thirty-six had negative cultures, and twenty-six had one or more types of bacteria in their urine. No correlation between symptomatology and pathological findings could be found. The authors stress the importance of constant vigilance, careful prophylaxis and effective therapy in all diabetic patients, as far as urinary tract infections are concerned.

Zahller, F. Marshall (Dept. of Pediat., Univ. of Nebraska Coll. of Med., Omaha, Nebr.): SPONTANEOUS HYPOGLYCEMIA IN CHILDHOOD. Nebraska M. J. 41:431-34, November 1956.

A classification of hypoglycemia, modified from Hartmann, is presented with a short discussion of carbohydrate metabolism as it is related to the various mechanisms of production of hypoglycemia. Also, differential diagnosis and methods of treatment are offered for those hypoglycemias of endocrine origin.

ORGANIZATION SECTION

OFFICERS AND MEMBERS OF COUNCIL, AMERICAN DIABETES ASSOCIATION, 1956-1957

SIXTH POSTGRADUATE COURSE

As previously announced, the American Diabetes Association will hold its Sixth Postgraduate Course in Diabetes and Basic Metabolic Problems Jan. 22, 23 and 24, 1958, in Atlanta, Georgia. Christopher J. McLoughlin, M.D., Atlanta, will serve as Director, with C. Raymond Arp, M.D., and Walter L. Bloom, M.D., Atlanta, as Associate Directors. This will be the first meeting of the Association to be held in the Southeast. Members and others who expect to attend may also wish to take advantage of this Georgia location in planning their winter vacations.

The preliminary program will be sent to the members of the Association as soon as possible. The Atlanta Biltmore will serve as headquarters hotel and registrants for the Course will be furnished with reservation cards. Those who plan to attend the Course are urged to register as soon as possible. All inquiries and applications should be addressed to the National Office of the American Diabetes Association.

FIFTH POSTGRADUATE COURSE

The Fifth Postgraduate Course in Diabetes and Basic Metabolic Problems offered by the American Diabetes Association was held in Columbus, Ohio, on Jan. 30-31 and Feb. 1, 1957. The Course was developed by the Committee on Professional Education of the American Diabetes Association, under the Chairmanship of Garfield G. Duncan, M.D., and was offered in cooperation with The Ohio State University Health Center.

The Director of the Course was George J. Hamwi, M.D., Associate Professor of Medicine and Head of the Division of Endocrinology and Metabolism, The Ohio State University College of Medicine; Head, Section of Endocrinology and Metabolism, University Hospital, Columbus, Ohio. Thomas P. Sharkey, M.D., Assistant Clinical Professor of Medicine, The Ohio State University College of Medicine and Consultant in Internal Medicine and Pathology, Miami Valley Hospital, Dayton, Ohio, was Associate Director.

A few comments from those who attended illustrate the success of the Course:

"I think the Course was exceedingly well done from the smallest details involving our comfort and the all important one of excellent speakers and subjects . . ."

"The most worthwhile and fruitful three days in the merry-go-round of medical meetings. Excellent balance between the laboratory and the clinic."

"An excellent, well-planned Course which was very stimulating to me personally. The subject material was pertinent at all times and, in general, well presented by the 'cream of the crop'. . ."

Physicians in attendance numbered 195. Two of this number have attended four courses; nineteen have attended three; and thirty-two have attended two.

The following is a registration breakdown for the

Fifth Postgraduate Course, as well as a comparison with the four previous Courses:

	1957	1956	1955	1954	1953
	Columbus	Dallas	Philadelphia	Rochester	Toronto
Members	83	82	135	83	93
Membership pending	55	52	23	24	23
Nonmembers	57	47	18	17	61
Total	195	181	176	124	177
Part-time registrants	1		2		
Graduate students			1	*	*
Fellows		2	3	*	*
Residents	16	10	10	*	*
Interns	8	4	5	*	*
Medical students	13	61	11	*	*
Councilors and Faculty	34	48	41	35	35
Guests	12	4	6		2
Others	25				
Total	109	129	79	35	37
Grand Total	304	310	255	159	214
	Additional Data				
Cancellations	12	12	15	25	5
Applications rejected due to limited facilities				47	92
Attendance at banquet	200	324	246	208	236

*Graduate Students, Fellows, Residents, Interns and Medical Students were not formally registered in 1954 and 1953.

THIRD CONGRESS, INTERNATIONAL DIABETES FEDERATION

The Third Congress of the International Diabetes Federation will be held in Düsseldorf, Germany, July 21-25, 1958. The First Congress met in Leyden, The Netherlands, in 1952, and the Second Congress at Cambridge England, in 1955.

Professor Dr. K. Oberdisse, Düsseldorf, has been designated Chairman and Priv. Doz. Dr. K. Jahnke, Düsseldorf, Organizing Secretary, of the Congress. An Advisory Committee, Third Congress, International Diabetes Federation of the American Diabetes Association, has been appointed. Its members are: Howard F. Root, M.D., Chairman, Frank N. Allan, M.D., Garfield G. Duncan, M.D., Francis D. W. Lukens, M.D., and Franklin B. Peck, Sr., M.D. Dr. Root is a Vice President of the International Diabetes Federation and a Past President of the American Diabetes Association. Franklin B. Peck, Sr., M.D., Secretary of the Association, has been named official Medical Delegate to the Congress. Mr. J. Richard Connelly, Executive Director, will serve as Lay Delegate. Additional information about the Congress will be published in future issues of this Journal.

RESEARCH FELLOWSHIPS AWARDED

The Committee on Research and Fellowships of the American Diabetes Association announces the award of three Research Fellowships for the 1957-58 academic year (July 1, 1957-June 30, 1958). Recipients include Wilson Randolph Tucker, M.D., Cincinnati, Ohio, Jefferson Earle White, Jr., M.D., Atlanta, Georgia, and Albert I. Winegrad, M.D., Philadelphia, Pennsylvania.

Dr. Tucker will study the experimental production of vascular lesions in the retina by administration of corticotropin or adrenal steroids, and the possible role of hyperlipemia in the genesis of such lesions. He will work with Chester Coggeshall, M.D., Chief, 1st Medical Service, St. Luke's Hospital, and Edwin F. Hirsch, M.D., Professor Emeritus of Pathology, University of Chicago.

Dr. White will work with Eugene A. Stead, Jr., M.D., Department of Medicine, Duke University School of Medicine, Durham, North Carolina. He will study the rate of utilization of ketone bodies (ketolysis) in the rat in varying states of endocrine and metabolic imbalance.

Dr. Winegrad will study the effect of various hexoses on unesterified fatty acids in plasma and in adipose tissue, in normals and in diabetics. He will work with Francis D. W. Lukens, M.D., The George S. Cox Medical Research Institute, Hospital of the University of Pennsylvania, Philadelphia.

The Committee on Research and Fellowships plans to award at least one Fellowship for the academic year 1958-59. The deadline for applications is Nov. 15, 1957. Requests for application forms and other inquiries should be addressed to Mr. J. Richard Connelly, Executive Director, who will forward the information to the Committee.

1958 LILLY AWARD

The second annual Lilly Award will be given at the Eighteenth Annual Meeting, June 21-22, 1958, in San Francisco. The following stipulations govern the contest for the award, which is supported by Eli Lilly and Company and consists of $1,000 and a medal.

Purpose: To recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Eligibility: Any investigator in an appropriate field of work closely related to diabetes who is less than forty

years of age on January 1 of the year in which the award is made. The research will not necessarily be judged in comparison to the work of more mature and experienced workers. The candidate should be a resident of the United States or Canada.

Nominations: Nominations for the award will be solicited from the members of the American Diabetes Association. Such nominations will be requested by repeated notices to be published in DIABETES. Names of nominees will be sent to the Chairman of the Committee on Scientific Awards and must be received before January 1 of the year of the award. The nomination should be accompanied by full information concerning the nominee's personality, training, and research work. Six copies of each item should be submitted. No member may send in more than one nomination. A list of the nominee's publications, if any, and six copies of the publication or manuscript for which the award is to be given should also accompany the nomination. At the discretion of the Committee on Scientific Awards, the award may be given for work published during the year prior to January 1 of the same year of the award. The nominee should be actively engaged at that time in the line of research for which the award is to be made.

Announcement: The name of the winner will be announced in the program of the Annual Meeting of the Association, and the award presented at that meeting. The winner, subject to the approval of the Committee on Scientific Programs, will be invited to present a paper on the subject of his work. Papers considered for the award must be submitted with the idea that they will be published in whole or in part in DIABETES if found acceptable to the Editor and/or Editorial Board. If the Committee should decide that no outstanding work has been presented for this consideration, the award will not be made.

Award: In addition to the monetary award and the medal, traveling expenses will be given to make it possible for the recipient to receive his award in person at the Annual Meeting.

NEW MEMBERS

Active

The following were elected as of April 1 and May 1, 1957:

California
- Loeffler, Robert W. — Los Banos

Georgia
- Hightower, John Allen — Brunswick

Illinois
- Klein, Samuel — Joliet
- McKay, Joseph Patrick — Bensenville
- Meredith, Paul A. — Chicago

Indiana
- Chroniak, Walter — Indianapolis

Iowa
- Locher, Robert Carroll — Cedar Rapids

Kentucky
- Harrison, Horace — Owensboro

Michigan
- Cubberley, Robert Bruce — Detroit
- Griffin, Robert M. — Muskegon
- Hulick, Archie George — Detroit

Minnesota
- Frethem, Allen A. — Rochester
- Molnar, George D. — Rochester

Missouri
- Alvi, Abdul Waheed — St. Louis

Nebraska
- Ahrens, Herbert George — Lincoln
- Gurnett, Thomas J. — Omaha
- Hervert, J. William — Lincoln

New Jersey
- Altschul, Frank Joseph — Long Branch
- Burt, Donald Peck — Morristown
- Failmezger, Theodore R. — Madison

New York
- Baird, Robert William — New York
- Roemmelt, John Carl — Elmira
- Rubenstein, Leo — Bronx

Ohio
- Anton, Anthony Thomas — Springfield
- Beck, William Clark — Oxford
- Bland, Rozier Earl — Columbus
- Cammarn, Maxine R. — Cleveland
- Devine, Walter Bernard — Zanesville
- Doan, Glenn B. — Greenfield
- Fish, Marvin — Columbus
- Gault, Ross M. — Portsmouth
- Griffin, William R. — Columbus
- Hannah, Gaston Beckett — Glendale
- Harris, Melvin William — Canton
- Kerns, Vemont D. — Circleville
- Kistler, Victor Niel — Lancaster
- Magness, John L. — Coshocton
- Mikesell, Hobart L. — West Liberty
- Mitchell, John A. — Newark
- Newell, Thomas Edmund — Dayton
- Nickerson, Irving Angus — Granville
- Ohlmacher, Joseph P. — Sandusky
- Pocock, Donald G. — Massillon
- Repasky, John G. — Akron
- Serbin, Richard A. — Columbus
- Sheets, Jerome Ritter — Portsmouth
- Williams, Thomas J. — Columbus
- Wilson, Harold J. — Columbus

Pennsylvania
- Blackburn, Lawrence F. — Greensburg
- Cohen, Jacob J. — Philadelphia
- Fabi, Mario Nestor — Scranton
- Mermelstein, Milton — McKeesport
- Schwartz, Norman Alvin — McKeesport
- Shaver, John C. — Pittsburgh
- Taylor, Arthur Ralph — Williamsport

Wu, Patrick Pih-Tsang	Philadelphia; and Free China
South Carolina	
Krueger, Kenneth W.	Hartsville
Tennessee	
Atkins, Leland L.	Memphis
Goldner, Fred	Nashville
Texas	
Moorman, Warren W.	Fort Worth
Virginia	
Shotton, Donald	Lynchburg
Washington	
Kilby, Ralph Allen	Spokane
West Virginia	
Smith, Charles Earl	Terra Alta
Other Countries	
Belgium	
Mahaux, Jacques	Brussels
Canada	
Johnson, Allen Campbell	Hamilton, Ontario
Levine, Benjamin P.	Outremont, Quebec

ADA CABLE ADDRESS

The American Diabetes Association has secured and registered "DIABETES NEWYORK" as a cable address in order to save correspondents money in transmitting messages.

This address cannot be used in the United States, Mexico and Canada because in these countries messages are considered telegrams and must be fully addressed. Messages from all other countries, however, can be cabled to "DIABETES NEWYORK." All companies handling cablegrams have been notified that this refers to the American Diabetes Association, Inc., 1 E. 45th St., New York 17, N. Y.

The five companies handling cables are: Western Union, All American, RCA, French and Globe Service.

NEWS OF AFFILIATE ASSOCIATIONS

The NEW JERSEY DIABETES ASSOCIATION (Clinical Society) held its annual dinner meeting May 15 in East Orange, N.J. The program included the following: "Case Report of Poliomyelitis in a Diabetic," by G. M. Knowles, M.D., Hackensack; "New Information on Oral Hypoglycemic Agents," by Herbert Kupperman, M.D., Newark; "Significance of the Quantity of Glycosuria in Relation to Age," by Alfred Gras, M.D., Newark; and "A Method of Teaching Self Injection of Insulin," by Everett O. Bauman, M.D., Newark. J. J. Torppey, M.D., Newark, Chairman of the Clinical Society, was in charge of the meeting.

The NEW YORK DIABETES ASSOCIATION (Clinical Society) held a meeting May 23 at the New York Academy of Medicine in New York City. The following papers were presented: "The Microangiopathy of Diabetes Mellitus," by Jørn Ditzel, M.D., Boston, with discussion by Milton Mendlowitz, M.D., New York City; "Acute Diabetic Cataract and Its Reversal—A Case Report," by Hans W. Neuberg, M.D., John H. Griscom, M.D., and Robert P. Burns, M.D., New York City, with discussion by George Wise, M.D., New York City; and "Current Status of Oral Hypoglycemic Agents," by Martin Goldner, M.D., New York. Irving Graef, M.D., Vice-Chairman of the Clinical Society, presided.

The VIRGINIA DIABETES ASSOCIATION presented a scientific program May 24 during the Seventh Annual Scientific Assembly of the Virginia Academy of General Practice held May 23-26 at Roanoke. Henry T. Ricketts, M.D., University of Chicago, spoke on "The Physiological Basis for Current Therapy of Degenerative Vascular Disease in Diabetes Mellitus." A panel discussion, "The Use of the Various Types of Insulin in the Management of Diabetes Mellitus, Including the Oral Hypoglycemic Agents," followed. Participants were Henry T. Ricketts, M.D., Chicago, Henry B. Mulholland, M.D., Charlottesville, Virginia, James M. Moss, M.D., Alexandria, Virginia, W. C. Salley, M.D., Norfolk, Virginia, and John C. Hortenstine, M.D., Winchester, Virginia. Norman Jolliffe, M.D., New York City, spoke on "The Modern Management of Obesity." Dr. Hortenstine was program chairman.

NEWS NOTES

NEW MEAL PLANNING PUBLICATIONS

"ADA Bland, Low-fiber Diabetic Diet" and "ADA Sodium Restricted Diabetic Diet" have recently been published and may be secured through the offices of the American Diabetes Association, 1 East 45 St., New York 17, N.Y. As announced in the July-August 1956 issue of DIABETES, "Meal Planning With Exchange Lists," a booklet prepared to help diabetics select foods for their meals, and Meal Plans No. 1 through No. 9 are also available.

The new material may be obtained at the same price as the other individual Meal Plans; namely, $.05 per single copy; $2.00 per 100 copies; $18.00 per 1,000 copies.

These meal planning leaflets were prepared by Committees of the American Diabetes Association and The American Dietetic Association in cooperation with the Chronic Disease Program of the U.S. Public Health Service.

Order forms are available on request.

TEACHING KIT RELEASED

The series of eleven film-strips and sound records from the teaching kit "Taking Care of Diabetes" has been re-released because of the continued interest in and demand for this material. This series was prepared cooperatively by the American Diabetes Association, Inc., The American Dietetic Association and the Public Health Service and originally released as a kit in 1951. All of the film-strips are in color; six are on medical nursing aspects and five on the dietary aspects of treatment. The titles are:

1. What is Diabetes? 2. Eating for Good Health. 3. Insulin and its Use. 4. Planning Good Meals. 5. Insulin Reaction. 6. Buying Good Food. 7. Tests in Diabetes. 8. Cooking Good Meals. 9. Diabetic Coma. 10. Care of Your Feet. 11. Selecting Meals for All Occasions.

The film-strips may be borrowed for short term loans from the Communicable Disease Center, Atlanta, Georgia, or from any of the Public Health Service Regional Offices in the following cities: Atlanta, Georgia; Charlottesville, Virginia; Chicago, Illinois; Dallas, Texas; Denver, Colorado; Kansas City, Missouri; New York City, New York; San Francisco, California.

The film-strips and records may be purchased from United World Films, Inc., 1445 Park Ave., New York 29, N.Y., at a price of $9.10 per individual film-strip and accompanying record.

BOOK BY DR. F. M. ALLEN AVAILABLE

All remaining copies of Dr. Frederick M. Allen's book on saltless diet entitled *Treatment of Kidney Diseases and High Blood Pressure,* 206 pages, published in 1925, are offered at nominal cost. The book is of historical interest for its pioneer advocacy of salt restriction for hypertension and related disorders. Terms to libraries: fifteen cents for postage; to individuals: one dollar per copy. Mail address with remittance to Miss A. Stacy, 1 East 84th St., New York 28, N. Y.

EMPLOYMENT OF DIABETICS IN THE FEDERAL SERVICE

The United States Civil Service Commission has released a leaflet entitled "Employment of Diabetics in the Federal Service." The Commission believes that "persons with *controlled* diabetes may be good employees and that it is good business to hire them. The four-page leaflet includes the following topics: "The Commission's Viewpoint," "Employment of Diabetics," and "Employment Criteria." Copies may be obtained from the United States Civil Service Commission, Washington, D.C.

PERSONALS

EDGAR C. BECK, M.D., Buffalo, EDWIN W. GATES, M.D., Niagara Falls, GEORGE F. KOEPF, M.D., Buffalo, ALFRED R. LENZNER, M.D., Buffalo, and J. FREDERICK PAINTON, M.D., Buffalo, participated in a post-graduate course on "Endocrine Disease" held at the University of Buffalo School of Medicine January 16-17. E. PERRY MCCULLAGH, M.D., Cleveland, was a member of the visiting faculty.

PIERO M. FOA, M.D., Chicago, and a team of scientists at Chicago Medical School, are engaged in experimentation relating to the oral hypoglycemic compounds. Their research is being supported by a United States Public Health Service grant of $11,500 a year. Originally awarded on a three-year basis, the grant has recently been renewed for another three-year period.

W. STANLEY HARTROFT, M.D., Ph.D., Chairman of the Department of Pathology, Washington University, St. Louis, Missouri, has been appointed to serve as a member of the Advisory Council of the Life Insurance Medical Research Fund for a four-year term.